University of Alberta

The Effects of Dobutamine in Hypoxic-Reoxygenated Newborn Piglets

By

Zakariya Al-Salam ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial

fulfillment of the requirements for the degree of Master of Science

in

Medical Sciences - Pediatrics

Edmonton, Alberta

Fall 2006

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-22218-8
Our file Notre référence
ISBN: 978-0-494-22218-8

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

ABSTRACT

Dobutamine is a commonly used inotrope to treat shock and hypotension that develop after the resuscitation of asphyxiated neonates. Using a neonatal swine model of hypoxia-reoxygenation, we examined the dose-response effect of dobutamine (5-20 µg/kg/min) on systemic and regional circulations, oxygen metabolism, platelet aggregatory function and markers of platelet activation. Piglets were subjected to hypoxia for 2h followed by reoxygenation for 4h. Dobutamine infusion was started after 2h of reoxygenation. High dose of dobutamine improved cardiac output, stroke volume and systemic oxygen delivery with no significant effect on heart rate, blood pressures, systemic vascular resistance, systemic oxygen consumption or regional circulations. Dobutamine (5-20 µg/kg/min) decreased pulmonary vascular resistance. However, high doses dobutamine infusion tended to cause platelet activation and aggregatory dysfunction.

In conclusion, dobutamine infusion in a neonatal swine model of hypoxia and reoxygenation caused a significant inotropic effect.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my supervisor Dr. Po-Yin Cheung for all the teaching, dedication, support and enthusiasm during my training. I also sincerely thank my supervisory committee members, Drs. Po-Yin Cheung, Susan Jacobs-Kaufman and Ernest Z. Phillipos for their support, advice and time.

I appreciate Drs. David Bigam and Scott Johnson for their surgical assistance, Dr. Sameh Abozaid for assistance during the experiment and Dr. Marwan Emara for his advice and technical support.

My gratitude also goes to all members of the Neonatal Sciences Laboratory, Grace Cheung, Corinne Cote, Jennifer Hugh, Judy Li and Dr. Raymond Lee for their help and cooperation, as well as to thank Aston Hugh for his computer assistance.

I would like to acknowledge the Neonatal-Perinatal Fellowship Program directors: Drs. Ernest Phillipos, Robert Lemke and Michael Narvey for all the teaching, advice and support during my training. I would like to thank all members of the Northern Alberta Neonatal Intensive Care Program for having me as Neonatal-Perinatal Clinical Research Fellow for all the teaching and support.

I am very grateful to my government, the Ministry of Higher Education and the Saudi Arabian Cultural Mission for giving me the opportunity, scholarship and the financial contribution.

I would like to acknowledge the financial contributions of Alberta Heritage Foundation for Medical Research and the Canadian Institutes of Health Research for operating funds.

At the end, I would like to acknowledge Drs. Mosaed El Fehaid, Muhammad A Majeed-Saidan, May Chehab and and Anne Tierney for all the support and teaching during my training at Riyadh Military Hospital, Saudi Arabia.

To my mother who did and continues to do anything for me

To my father who believes in me

To my wife Tahani for all her love, patience and support

CONTENTS

Page

CHAPTER 1: Neonatal asphyxia and reoxygenation

The clinical aspect of neonatal asphyxia..2

Responses to asphyxia..4

References..11

CHAPTER 2: Cardiovascular and platelet pathophysiology during neonatal asphyxia and reoxygenation

Cardiovascular physiology..15

Systemic blood pressure and blood flow..16

Platelet physiology..17

Hemostatic pathways..18

Platelet aggregation: signal transduction..19

Effects of hypoxia-reoxygenation on the myocardium..21

Effects of hypoxia-reoxygenation on systemic, pulmonary and regional vasculatures....25

Platelets pathology..26

Effect of hypoxia-reoxygenation on platelets...27

References..29

CHAPTER 3: Hemodynamic support after neonatal asphyxia

Management of neonatal asphyxia 39

Hemodynamic support in neonatal asphyxia..40

References..47

CHAPTER 4: Dobutamine

Chemistry 54

Pharmacokinetics..........55

Therapeutic uses..........55

Cardiovascular effects of dobutamine in neonates..........55

Dobutamine and regional blood flow..........56

Dobutamine and oxygen consumption..........57

Dobutamine and platelet..........58

References..........62

CHAPTER 5: Animal models of hypoxia and reoxygenation

Introduction 67

Types of neonatal asphyxia model..........67

Similarities between piglets and human..........69

Hypoxia and reoxygenation models in newborn piglets..........70

Conclusions..........72

References..........74

CHAPTER 6: The hemodynamic effects of dobutamine during reoxygenation after hypoxia: a dose-response study in newborn pigs

Abstract..........80

Introduction..........82

Methods..........83

Statistical analysis..........87

Results..........88

Discussion.....92

Conclusions.....97

References.....117

CHAPTER 7: The effect of dobutamine on platelet aggregatory function in newborn piglets with hypoxia and reoxygenation

Abstract.....125

Introduction.....127

Methods.....128

Statistical analysis.....132

Results.....133

Discussion.....135

Conclusions.....138

References.....147

CHAPTER 8: The myocardial matrix metalloproteinase-2 and -9 and nitrotyrosine levels in newborn piglets with hypoxia and reoxygenation during dobutamine infusion

Abstract.....151

Introduction.....153

Methods.....154

Statistical analysis.....157

Results.....157

Discussion.....158

Conclusions.....160

References...164

CHAPTER 9: Conclusions and implications

....170

LIST OF TABLES

Page

1-1: Antepartum risk factors associated with neonatal depression and asphyxia............7

1-2: Intrapartum risk factors associated with neonatal depression and asphyxia............8

1-3: Neonatal risk factors associated with neonatal depression and asphyxia................9

1-4: Common short-term consequences of asphyxia...10

5-1: Commonly used methods of inducing perinatal/neonatal asphyxia........................73

6-1: Acid-base balance in newborn piglets during hypoxia, reoxygenation and dobutamine infusion...98

6-2: Plasma lactate (mmol/L) in newborn piglets during hypoxia, reoxygenation and dobutamine infusion...99

7-1: Heart rate and mean systemic arterial pressure at baseline, 2h of hypoxia, 2h of reoxygenation and 2h of dobutamine infusion...140

7-2: Arterial blood pH, partial pressure of oxygen and bicarbonate concentration at baseline, 2h of hypoxia, 2h of reoxygenation and 2h of dobutamine infusion............141

7-3: Collagen-stimulated whole blood aggregatory responses at baseline and end of hypoxia-reoxygenation (2h of dobutamine or saline administration).......................142

7-4: Plasma concentrations of TxB_2, cAMP, cGMP and nitrotyrosine at 2h of dobutamine infusion...143

LIST OF FIGURES

Page

2-1: Platelet activation and inhibitory pathways..28

4-1: Chemical structure of dobutamine...61

6-1: The effect of dobutamine infusion in cardiac index in newborn piglets during hypoxia and reoxygenation...100

6-2: The effect of dobutamine infusion in stroke volume index in newborn piglets during hypoxia and reoxygenation...101

6-3: The effect of dobutamine infusion in heart rate in newborn piglets during hypoxia and reoxygenation...102

6-4: The effect of dobutamine infusion in mean systemic arterial pressure in newborn piglets during hypoxia and reoxygenation..103

6-5: The effect of dobutamine infusion in mean pulmonary arterial pressure in newborn piglets during and hypoxia reoxygenation..104

6-6: The effect of dobutamine infusion in pulmonary arterial pressure/systemic arterial pressure ratio in newborn piglets during hypoxia and reoxygenation......................105

6-7: The effect of dobutamine infusion in systemic vascular resistance index in newborn piglets during hypoxia and reoxygenation..106

6-8: The effect of dobutamine infusion in estimated pulmonary vascular resistance index in newborn piglets during hypoxia and reoxygenation.....................................107

6-9: The effect of dobutamine infusion in systemic oxygen delivery in newborn piglets during hypoxia and reoxygenation..108

6-10: The effect of dobutamine infusion in systemic oxygen consumption in newborn piglets during hypoxia and reoxygenation........109

6-11: The effect of dobutamine infusion in systemic oxygen extraction ratio in newborn piglets during hypoxia and reoxygenation........110

6-12: The effect of dobutamine infusion in carotid arterial flow index in newborn piglets during hypoxia and reoxygenation........111

6-13: The effect of dobutamine infusion in carotid arterial oxygen delivery in newborn piglets during hypoxia and reoxygenation........112

6-14: The effect of dobutamine infusion in superior mesenteric arterial flow index in newborn piglets during hypoxia and reoxygenation........113

6-15: The effect of dobutamine infusion in superior mesenteric arterial oxygen delivery in newborn piglets during hypoxia and reoxygenation........114

6-16: The effect of dobutamine infusion in renal arterial flow index in newborn piglets during hypoxia and reoxygenation........115

6-17: The effect of dobutamine infusion in renal arterial oxygen delivery in newborn piglets during hypoxia and reoxygenation........116

7-1: Platelet counts at baseline and end of hypoxia-reoxygenation (2h of dobutamine or saline infusion)........144

7-2: Collagen-stimulated whole blood aggregatory responses........145

7-3: Temporal changes in the plasma MMP-9 and MMP-2 activities........146

8-1: Left ventricular matrix metalloproteinase-2 activity in control and dobutamine-treated groups after hypoxia and reoxygenation........161

8-2: Left ventricular matrix metalloproteinase-9 activity in control and dobutamine-treated groups after hypoxia and reoxygenation……………………………………..162

8-3: Left ventricular nitrotyrosine concentration in control and dobutamine-treated groups after hypoxia and reoxygenation……………………………………………………..163

ABBREVIATIONS

ANOVA: Analysis of variance

CA: Carotid artery

cAMP: Cyclic adenosine 3',5' monophosphate

cGMP: Cyclic guanosine 3',5' monophosphate

CI: Cardiac index

CVP: Central venous pressure

ECG: Electrocardiogram

FI: Flow index

FiO$_2$: Fractional inspired oxygen concentration

Fr: French sizing of catheters

H-R: Hypoxia-reoxygenation

i.v.: Intravenous

LV: Left ventricle

MMP: Matrix metalloproteinase

NADH: reduced nicotinamide adenine dinucleotide

NO: Nitric oxide

PA: Pulmonary artery

PaCO$_2$: Arterial partial pressure of carbon dioxide

PaO$_2$: Arterial partial pressure of oxygen

PAP: Pulmonary arterial pressure

PVRI: Pulmonary vascular resistance index

RA: Renal artery

SaO$_2$: Arterial hemoglobin oxygen saturation

SAP: Systemic arterial pressure

SMA: Superior mesenteric artery

SVI: Stroke volume index

SVRI: Systemic vascular resistance index

SysDO$_2$: Systemic oxygen delivery

SysEO$_2$: Systemic oxygen extraction ratio

SysVO$_2$: Systemic oxygen consumption

TxB$_2$: Thromboxane B$_2$

CHAPTER 1

Neonatal asphyxia and reoxygenation

The clinical aspect of neonatal asphyxia

Asphyxia is a condition of impaired gas exchange that results in hypoxemia, hypercapnia and metabolic acidosis (1,2). It is a major cause of perinatal and neonatal morbidity and mortality. It is a common problem worldwide. Birth asphyxia affects about 4-9 million neonates every year and accounts for 24% to 61% of all perinatal mortality (3). Approximately 1.2 million neonates die every year due to birth asphyxia (4).

There are many risk factors that are associated with neonatal asphyxia. Recognizing these factors is very important to prevent and treat neonatal asphyxia promptly. They include antepartum risk factors (e.g. very young or old maternal age, maternal illness, drugs and no prenatal care) (Table 1-1), intrapartum risk factors (e.g. problems with placenta, umbilical cord, amniotic fluid and the fetus) (Table 1-2) and neonatal risk factors (e.g. cardiorespiratory diseases) (Table 1-3). Affected neonates may have more than one risk factor at the same time.

Due to the medicolegal aspect of birth asphyxia and subsequent development of neonatal encephalopathy, the American College of Obstetricians and Gynecologists and the International Cerebral Palsy Task Force have published strict criteria to define acute intrapartum hypoxic event (5). These criteria must be met before an intrapartum hypoxic-ischemic insult can be labeled as a cause of moderate to severe neonatal encephalopathy. They are: 1) evidence of metabolic acidosis in fetal umbilical cord arterial blood obtained at delivery (pH less than 7 and base deficit of 12 mmol/L or more), 2) early onset of severe or moderate neonatal encephalopathy in infants born at 34 or more weeks' gestation, 3) cerebral palsy of spastic quadriplegic or dyskinetic type, and 4) exclusion of other identifiable etiologies, such as trauma, coagulation disorders, infectious conditions, or genetic disorders. Other criteria that together suggest the intrapartum timing include: 1) a sentinel (signal) hypoxic event occurring immediately before or during labor, 2) a sudden and sustained fetal bradycardia or the absence of fetal heart rate variability in the presence of persistent late or persistent variable decelerations, usually after a sentinel hypoxic event when the pattern was previously normal, 3) Apgar scores of 0-3 beyond 5 minutes of life, 4) onset of multisystem involvement within 72 hours of birth, 5) early imaging study showing the evidence of acute nonfocal cerebral abnormality.

Neonatal morbidities due to asphyxia are common. Table 1-4 shows the short-term consequences of asphyxia. The central nervous system is the most frequently involved system (72%) in neonatal asphyxia, followed by renal (42%), cardiac (29%), gastrointestinal (29%) and pulmonary system (26%) (6). More than one system can be involved at the same time resulting in multiorgan dysfunction. Involvement of one or more organs occurs in 82% of the neonates (7).

One of the serious consequences of neonatal asphyxia is hypoxic-ischemic encephalopathy which is also associated with significant mortality and morbidity (7). Ten percent of infants with moderate hypoxic-ischemic encephalopathy die and this can rise to 60% in the severe form (8). Survivors may suffer from short-term morbidities, major neurological disabilities and developmental delay (7,9). Beside hypoxic-ischemic encephalopathy, asphyxia can lead to intracranial hemorrhage, cerebral edema and seizures (6).

Myocardial dysfunction and hypotension are well-known cardiovascular complications which could be worsened by the presence of respiratory failure secondary to meconium aspiration and pulmonary hypertension of the newborn. Acute renal failure in asphyxiated neonates can be due to acute tubular or cortical necrosis. Necrotizing enterocolitis is another serious gastrointestinal complication that needs intensive medical and surgical interventions. Finally, platelet dysfunction, thrombocytopenia and disseminated intravascular coagulation can lead to thrombo-embolic complications or hemorrhage following asphyxia.

Responses to asphyxia

The main function of the cardiovascular system is to maintain blood flow to meet the metabolic demand of the body. Hypoxemia and asphyxia cause a fall in heart rate and combined ventricular output (10). This results in a circulatory centralization in favor of the brain, heart, and adrenals which occurs at the expense of almost all peripheral organs, particularly the lungs, carcass, skin and scalp. However, when fetal hypoxemia is severe, the circulatory centralization can not be maintained and the fetus experiences severe brain damage and dies, unless immediate resuscitation is provided.

Regional adjustments in vascular tone can be achieved by autoregulation mechanisms and neurohumoral input. Organs like the heart and the brain have a good ability for autoregulation, while other organs like the skin and muscle have poor autoregulation. Skin, muscles, splanchnic organs and kidneys have extensive neural and humoral input. In case of decreased blood flow or hypoxia, the myogenic response results in vasodilation of the blood vessel, which supplies the heart and brain whereas neurohumoral (catecholamines, endothelin-1 and angiotensin II) input leads to vasoconstriction in other organs.

Hematological adjustments during the acute phase of hypoxia include rightward shift of the oxygen dissociation curve due to the accumulation of lactic acid (decreased pH) and other metabolites. Rightward shift, equates to a lower hemoglobin affinity for oxygen and thus an increased unloading of oxygen to the tissue. Chronic hypoxemia can lead to an increase in 2,3-diphosphoglycerate, which in turn decreases the affinity of hemoglobin for oxygen. Hemoglobin synthesis can be stimulated by chronic hypoxemia due to enhanced erythropoietin production, which could be regulated by hypoxia-inducible factor-1α. The latter is a transcription factor that is increased by low cellular oxygen tension (11). Although a high hemoglobin concentration can increase oxygen content in the blood, it can also increase blood viscosity and impede blood flow.

During the early phase of acute hypoxemia, oxygen delivery is low while tissue oxygen extraction is high. If the oxygen supply to organs falls to a critical level, tissues must utilize anaerobic metabolism as an alternative mechanism to maintain cellular energy. This results in an accumulation of lactic acid and subsequent impairment of organ function (12,13). In chronic hypoxemia, oxygen tissue consumption decreases following a decrease in the metabolic rate (14). Chronic hypoxia and decreased metabolic rate impair fetal growth. It has been estimated that 30-40% of oxygen consumption used by fetal lamb is used for fetal growth. Intrauterine growth retardation and low birth weight infants are well known complications associated chronic intrauterine hypoxia (15).

The "developmental origins of adult disease" hypothesis, often called the "Barker hypothesis", states that adverse influences early in development, and particularly during intrauterine life, can result in permanent changes in physiology and metabolism, which are associated with an increased risk for diseases in adulthood (16). Low birth weight due to intrauterine events is associated with an increased incidence of cardiovascular, metabolic and other diseases later in life (16). Epidemiological studies in humans have shown that chronic fetal hypoxia secondary to hypobaric hypoxia of high altitude is associated with impaired intrauterine growth, reduced birth weight and asymmetric growth retardation (17). In rats, chronic hypoxia during pregnancy causes the same effect and leads to abnormalities in the cardiovascular function of adult offsprings. This is thought to be due to a combination of fetal hypoxia and maternal malnutrition. However, hypoxia alone during early development can program the cardiovascular system and, more specifically, the blood vessels (18). In rats, prenatal hypoxia but not nutrient restriction impairs endothelium-dependent relaxation in the mesenteric circulation of adult offsprings (18).

Asphyxia is a major cause of morbidity and mortality worldwide. It may result in serious multiorgan dysfunction and long-term sequelae such as cerebral palsy. Intrauterine growth retardation, as result of intrauterine chronic hypoxia, has been linked to major chronic illness in adulthood. Little is known regarding the short-term and long-term effects of neonatal asphyxia on the cardiovascular system. More studies are needed in order to prevent and treat the consequences of neonatal asphyxia.

Table 1-1: Antepartum risk factors associated with neonatal depression and asphyxia (19).

- Maternal diabetes
- Pregnancy-induced hypertension
- Chronic hypertension
- Chronic renal failure
- Chronic illnesses
  - Cardiovascular
  - Thyroid
  - Neurological
  - Pulmonary
  - Renal
- Anemia or isoimmunization
- Previous fetal or neonatal death
- Bleeding in second or third trimester
- Maternal infections
- Diminished fetal activity
- Polyhydramnios
- Oligohydramnios
- Premature rupture of membranes
- Post-term gestation
- No prenatal care
- Age < 16 or > 35 years
- Multiple gestations
- Size-date discrepancy
- Drug therapy, e.g.:
  - Lithium carbonate
  - Magnesium
  - Adrenergic-blocking drugs
- Maternal substance abuse
- Fetal malformation

Table 1-2: Intrapartum risk factors associated with neonatal depression and asphyxia (19).

Emergency cesarean section
Forceps or vacuum-assisted delivery
Breech or other abnormal presentation
Premature labor
Precipitous labor
Chorioamnionitis
Prolonged rupture of membranes (> 18 hours before delivery)
Prolonged labor (>24 hours)
Prolonged second stage of labor (> 2 hours)
Fetal bradycardia
Non reassuring fetal heart rate patterns
Use of general anesthesia
Uterine tetany
Narcotics given to mother within 4 hours of delivery
Meconium-stained amniotic fluid
Prolapsed cord
Abruptio placenta
Placenta previa

Table 1-3: Neonatal risk factors associated with neonatal depression and asphyxia (19).

Central nervous system	
	Meningoencephalitis
	Congenital muscular dystrophy
	Spinal muscular dystrophy
	Intracranial hemorrhage
	Head trauma
Respiratory system	
	Severe respiratory distress syndrome
	Persistent fetal circulation
	Congenital diaphragmatic hernia
	Airway obstructions
	Congenital pulmonary malformations
	Apnea
Cardiovascular system	
	Congenital heart diseases (cyanotic, obstructive)
Miscellaneous	
	Drug intoxication
	Child abuse
	Sepsis

Table 1-4: Common short-term consequences of asphyxia (6,19).

- Central nervous system (72%)
  - Cerebral hemorrhage
  - Cerebral edema
  - Hypoxic-ischemic encephalopathy
  - Seizures
- Cardiovascular system (29%)
  - Myocardial failure
  - Papillary muscle necrosis
  - Persistent fetal circulation
- Respiratory system (26%)
  - Delayed onset of respiration
  - Respiratory distress syndrome
  - Meconium aspiration syndrome
- Renal system (42%)
  - Cortico/ tubular/ medullary/ necrosis
- Gastrointestinal system (29%)
  - Necrotizing enterocolitis
- Hematological system
  - Disseminated intravascular coagulation
  - Thrombocytopenia
  - Platelet dysfunction

References

1. Low JA 1997 Intrapartum fetal asphyxia: definition, diagnosis, and classification. Am J Obstet Gynecol 176:957-959
2. Bax M, Nelson KB 1993 Birth asphyxia: a statement. World Federation of Neurology Group. Dev Med Child Neurol 35:1022-1024
3. Ellis M 2000 Improving newborn health in developing countries. In: Costello A, Manandhar D (eds) Birth asphyxia in developing countries: epidemiology, sequelae and prevention. Imperial College Press, London, pp 233-272
4. Black RE, Morris SS, Bryce J 2003 Where and why are 10 million children dying every year? Lancet 361:2226-2234
5. Hankins GD, Speer M 2003 Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. Obstet Gynecol 102:628-636
6. Martin-Ancel A, Garcia-Alix A, Gaya F, Cabanas F, Burgueros M, Quero J 1995 Multiple organ involvement in perinatal asphyxia. J Pediatr 127:786-793
7. Shah P, Riphagen S, Beyene J, Perlman M 2004 Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed 89:F152-F155
8. Shankaran S, Woldt E, Koepke T, Bedard MP, Nandyal R 1991 Acute neonatal morbidity and long-term central nervous system sequelae of perinatal asphyxia in term infants. Early Hum Dev 25:135-148
9. Robertson CM, Finer NN, Grace MG 1989 School performance of survivors of neonatal encephalopathy associated with birth- asphyxia at term. J Pediatr 114:753-760

10. Rudolph AM, Heymann MA 1976 Cardiac output in the fetal lamb: the effects of spontaneous and induced changes of heart rate on right and left ventricular output. Am J Obstet Gynecol 124:183-192

11. Semenza GL 1994 Regulation of erythropoietin production. New insights into molecular mechanisms of oxygen homeostasis. Hematol Oncol Clin North Am 8:863-884

12. Stein JC, Ellsworth ML 1993 Capillary oxygen transport during severe hypoxia: role of hemoglobin oxygen affinity. J Appl Physiol 75:1601-1607

13. Jacobs R, Owens JA, Falconer J, Webster ME, Robinson JS 1988 Changes to metabolite concentration in fetal sheep subjected to prolonged hypobaric hypoxia. J Dev Physiol 10:113-121

14. Richardson BS, Bocking AD 1998 Metabolic and circulatory adaptations to chronic hypoxia in the fetus. Comp Biochem Physiol A Mol Integr Physiol 119:717-723

15. Jacobs R, Robinson JS, Owens JA, Falconer J, Webster ME 1988 The effect of prolonged hypobaric hypoxia on growth of fetal sheep. J Dev Physiol 10:97-112

16. Barker DJ 2006 Adult consequences of fetal growth restriction. Clin Obstet Gynecol 49:270-283

17. Gardner DS, Powlson AS, Giussani DA 2001 An in vivo nitric oxide clamp to investigate the influence of nitric oxide on continuous umbilical blood flow during acute hypoxaemia in the sheep fetus. J Physiol 537:587-596

18. Williams SJ, Hemmings DG, Mitchell JM, McMillen IC, Davidge ST 2005 Effects of maternal hypoxia or nutrient restriction during pregnancy on endothelial function in adult male rat offspring. J Physiol 565:125-135

19. Bloom RS 2006 Delivery room resuscitation of newborn. In: Martin RM, Fanaroff AA (eds) Neonatal-perinatal medicine: diseases of the fetus and infant. Mosby Inc, St. Louis, pp 416-439

CHAPTER 2

Cardiovascular and platelet pathophysiology during neonatal asphyxia and reoxygenation

Cardiovascular physiology

The cardiac innervation is derived from sympathetic and parasympathetic nervous systems. Sympathetic stimulation of the heart leads to increases in heart rate (positive chronotropy) and force of contraction (positive inotropy) while stimulation of the heart by the parasympathetic nervous system leads to the opposite, decreases in heart rate (negative chronotropy) and force of contraction (negative inotropy). Blood vessels receive innervations from sympathetic innervation which upon activation will lead to vasoconstriction. The adrenal gland secretes the catecholamine epinephrine (adrenaline) that acts on the heart and increases myocardial contractility and heart rate. Cardiovascular function is also controlled by hormones such as angiotensin II, aldosterone, antidiuretic hormone, and others. These hormones affect vasomotor tone regulation, salt and water balance and, ultimately, control blood pressure and cardiac output.

Cardiac output, defined as the volume of blood ejected per unit time, is the product of heart rate and stroke volume. If heart rate increases, that will lead to an increase in cardiac output. However, the increase in cardiac output due to tachycardia is limited. At very high heart rate, the end-diastolic volume and hence the stroke volume will decease due to the short time to re-fill the heart during the diastolic phase. Stroke volume depends on the left ventricular volume and myocardial fiber shortening. The latter depends on the preload (the venous return, end-diastolic volume), force of contraction and after-load (blood pressure and vascular resistance). Starling's law (energy of contraction is proportional to the initial length of the cardiac muscle fiber) dictates that if the end-diastolic volume increases, stroke volume and therefore cardiac output will increase. The failing heart however can not increase stroke volume in response to an increase in the end-diastolic volume. Subsequently, end-diastolic volume and pressure will increase and lead to symptomology of heart failure. Beside these intrinsic properties of the heart, there are many physiological and pathological conditions that can affect the cardiac output. Exercise, high fever, infections, pregnancy, thyrotoxicosis, sympathomimetic drugs and anxiety are some of the conditions that can increase the cardiac output. Hypothermia, hypothyroidism, sympatholytic drugs and arrhythmias can decrease cardiac output.

Systemic blood pressure and blood flow

What is normal blood pressure in neonates? It is probably one of the most difficult questions that challenge every practitioner in the care of critically ill neonates. It could be related to many factors. First, blood pressure varies a lot according to birth weight, gestational and postnatal ages (1,2). Second, it is difficult to extrapolate blood pressure values measured in neonates admitted to neonatal intensive care unit and make recommendation of the normal ranges (2).

In the management of neonatal hypotension, most clinicians aim to maintain normal blood pressure in order to maintain normal flow and tissue perfusion to meet the metabolic demand of various organs. However, it is speculative to derive a direct relationship between blood pressure and blood flow, both systemically and regionally to a specific organ. By the principle of Ohm's law (flow = pressure ÷ resistance), blood pressure is directly proportional to resistance. Therefore the blood pressure may rise because the resistance is high, but that will be at the expense of actual blood flow. Clinically, Kluckow et al found a significant but weak correlation between systemic arterial pressure and cardiac output measured by superior vena caval flow (3,4).

Therefore, using systemic arterial pressure alone as a measure of cardiac output is unreliable and a direct measurement of cardiac output is needed.

It is also important to mention that organ perfusion depends on the hydrostatic pressure in order to function properly. Cerebral perfusion pressure equals mean arterial pressure minus intracranial pressure and central venous pressure. In general, cerebral perfusion pressure approximates 20-25 mmHg. Interestingly, it has been shown that cerebral intravascular oxygenation correlates with mean arterial pressure in critically ill premature infants (5). In addition, the glomerular filtration rate (glomerular function) depends on the hydrostatic and oncotic pressures on both the blood vessels and renal tubules. The net pressure should be high enough to force fluid out of blood vessel and into the Bowman's capsule. Therefore, hypotension may lead to cerebral ischemia and acute renal failure.

Platelet physiology

Platelets are critical components of the hemostatic cascade. They have a unique structure (6). They are the smallest of the three blood cell types (the others: red and white blood cells), with diameters of approximately 2-3 μm, and concentrations of 150-450 x 10^9/L. They have a half-life of 4-10 days. Their production (thrombopoeisis) is stimulated by thrombopoeitin. Thrombopoeitin acts on committed stem cells in the bone marrow, causing them to develop into large multinucleated megakaryocytes, which eventually break apart releasing platelets as cellular fragments into the bloodstream. The platelets have no nuclei, but are full of granules, which contain the many factors necessary for hemostasis.

Hemostasis is divided into two stages: primary hemostasis and secondary hemostasis. Primary hemostasis is marked by vasoconstriction and the construction of a "platelet plug", and secondary hemostasis concludes the process by the formation of a blood clot. It is by these interacting and overlapping mechanisms that the body is able to minimize the amount of blood lost following vascular injury.

Hemostatic pathways

Hemostasis is triggered by vascular injury which results in the exposure of collagen in the extracellular matrix to platelets in the blood. When collagen is exposed to platelets, it causes them to adhere to the site of injury (Figure 2-1). This adhesion is facilitated by von Willebrand factor (vWF). Subsequently, platelet activation takes place and results in the local release of adenosine diphosphate (ADP), thromboxane A_2 (TxA_2) and serotonin. The release of ADP and TxA_2 will result in the recruitment and activation further platelets, which allow for the accumulation of a platelet monolayer and the beginning of platelet plug formation (6). Furthermore, serotonin and TxA_2 promote vasoconstriction of the blood vessel. This, together with the adhesion and aggregation of platelets, comprises primary hemostasis. In secondary hemostasis, prothrombin is converted to thrombin in the coagulation cascade. In its active form, thrombin serves as a proteolytic enzyme, cleaving molecules of fibrinogen into fibrin monomers. Fibrin initially forms a loose mesh, but certain plasma factors cause the formation of covalent cross-links. These covalent cross-links convert fibrin to a dense aggregation of fibers. Platelets and red blood cells become caught in this meshwork of fiber, and lead to the formation of a blood clot, which is the final step in hemostasis (6).

The above processes must be tightly controlled to ensure that clotting is not inappropriately initiated. Certain receptors on the surface of platelets are responsible for controlling the specificity of reactions. For example, there are receptors for the following agonists: collagen, vWF, ADP, epinephrine, and TxA_2 (7). Binding of these agonists to their receptors will transmit extracellular signals into the cell, which can cause a number of responses including granular secretion, aggregation, ionic influx and reorganization of the cytoskeleton.

Platelet aggregation: signal transduction

There are two collagen receptors on platelets: integrin $\alpha_2\beta_1$ and glycoprotein (GP) VI, as well as two receptors that bind vWF: GPIb-IX-V and integrin $\alpha_{IIb}\beta_3$. Agonistic binding to integrin $\alpha_{IIb}\beta_3$ causes the receptors to cluster, which leads to conformational changes in their cytoplasmic domains which in part will thereby trigger signaling cascades. When collagen binds with GP VI, clustering of GP VI activates an enzyme called Syk (tyrosine kinase) which will phosphorylate and activate phospholipase Cγ, leading to the secretion of ADP and the production and release of TxA_2. As a result, ADP and TxA_2 go on to bind to activate further platelets (8).

Adenosine diphosphate also activates two (G-protein-coupled receptors) receptors: $P2Y_1$ and $P2Y_{12}$ (Figure 2-1). In the resting state, the receptors are bound to the inactive G-protein, which has GDP associated with it. When ADP binds with the receptor, the G-protein becomes active and converts GDP to GTP. As a result, GTP interacts with adenylyl cyclase and leads to its suppression. Adenylyl cyclase is the enzyme that synthesizes cyclic adenosine monophosphate (cAMP) from ATP. Cyclic AMP functions as a second messenger. Raising the intracellular concentration of cAMP causes the general inhibition of their response to agonists. The G-protein family, however, suppresses adenylyl cyclase and subsequently decreases cAMP, relieving the block on further platelet signaling. In this way, cAMP functions as a check on impromptu platelet activation.

Nitric oxide, a product of the enzyme nitric oxide synthase, activates soluble guanylate cyclase, which results in an increase in intracellular cyclic guanosine monophosphate (cGMP) levels (9). Increased cytoplasmic levels of cGMP reduce Ca^{2+} fluxes in platelets, therefore having an inhibitory effect on aggregation.

Platelets possess adrenoreceptors (α and β) and are therefore affected by epinephrine. For example, when epinephrine binds to α_2 receptors on platelets, there is an intracellular decrease in cAMP levels through the GTP-adenylate cyclase pathway (9). This results in the potentiation of platelets to agonists and to further platelet activation.

Another effect of platelet adhesion and aggregation by collagen is the release of a class of enzymes called the matrix metalloproteinases (MMPs). They are a family of zinc-containing endopeptidases that are involved in various physiological and pathological conditions (10). Their major source is from the leukocytes. They contribute to tissue remodeling and cell migration through cleavage of matrix proteins and adhesion molecules. Two MMPs, MMP-2 and MMP-9, are specific to platelet function and are produced as inactive precursors in the cytoplasm (as pro-MMP-2 and pro-MMP-9) (11-13). Platelet adhesion by vWF and aggregation by collagen causes the release of cytoplasmic pro-MMP-2, which is then activated at the surface membrane. Matrix metalloproteinase-2 is thought to be involved in the modification of major platelet GP receptors, GP Ib and GP IIb/IIIa. Matrix metalloproteinase-2 primes platelets for adhesion and aggregation (11), whereas MMP-9 has an inhibitory role on the platelet and counteracts the platelet-aggregatory effects of MMP-2 (12,13). Matrix metalloproteinase-2 is also offset by prostacyclin I_2 and nitric oxide which down-regulates the production of pro-MMP-2.

Effects of hypoxia-reoxygenation on the myocardium

There is a limited amount of literature on the neonatal myocardial dysfunction secondary to asphyxia. This may be explained by the fact that most research focuses on the neurological effects of neonatal asphyxia. In addition, the neonatal myocardial involvement is often underestimated or thought to be transient with no implication in the outcome of the infant as long as the blood flow to the brain is not affected.

The cardiovascular system is unique in its development. First, *in utero* it has to function to support the growth and development of various organs in a relatively hypoxic environment. It also handles the acute hypoxic event of labor and delivery and switches from a parallel to a series type of circulation immediately after birth. During the development of the fetus, hypoxic insults can occur at any time and are usually tolerated. However, when the insult is too long or too severe, it can result in a serious damage to the developing fetus and can lead to death.

There is some evidence from animal studies suggesting that the neonatal myocardium is more resistant to hypoxia and reoxygenation/reperfusion than the adult myocardium (14,15). The normal fetal partial pressure of umbilical venous oxygen (oxygenated blood coming from placenta) is 30-37 mmHg, while in the adult the arterial partial pressure of oxygen is 80-100 mmHg. The fetus compensates for this by several mechanisms which include: 1) higher fetal hemoglobin concentration and higher oxygen affinity than that found with adult hemoglobin, 2) leftward shift of oxygen-hemoglobin dissociation curve and 3) higher cardiac output and thus systemic oxygen delivery per body weight. The fetal and neonatal myocardium are also more resistant to hypoxia than adult type myocardium (16,17). This could be explained by 1) higher rate of anaerobic glycolysis (18), and 2) greater glycogen storage in the immature myocardium (19). This may be important in handling the amount stress related to birth (hypoxia and respiratory and metabolic acidosis) that every newborn normally goes through.

Acidosis decreases myocardial contractility in the isolated myocardium. However, in intact animals, acidosis ($pH<6.8$) or hypoxemia ($PaO_2<25$ mmHg) alone does not depress myocardial function (20,21). On the other hand, the combination of acidosis and hypoxemia can decrease myocardial function in intact animals (22). The preserved cardiac function in intact animals seems to be related to a compensatory mechanism by the sympathoadrenal system and calcium influx during acidosis. Disrupting or blocking these two processes can worsen cardiac function (23,24).

The immature heart is also more resistant to ischemia, where there is a lack of substrate delivery, accumulation of metabolites, as well as hypoxemia and acidosis (25). However, when the ischemic insult persists for a long period of time, myocardial damage and cell death will ultimately occur. The tolerance of the immature heart to ischemia seems to be particularly important in the event of resuscitation and surgical correction of congenital heart anomalies in the neonatal period, then recovery and tolerance are better than that in the adult heart.

The neonatal myocardium is also more resistant to reperfusion/reoxygenation injury than the adult myocardium (26). Immediately after reoxygenation, cardiac output recovers due to increases in heart rate and stroke volume (27). However, this is usually followed by myocardial dysfunction (myocardial stunning) with decreased stroke volume (27). The heart rate may increase to compensate for the low cardiac output state. The mechanism of myocardial stunning may be related to oxidative stress (28). Oxygen free radicals such as superoxide, formed very early during reperfusion/reoxygenation, can lead to myocardial dysfunction (28). Furthermore, peroxynitrite, the reactive nitrogen species, has also been shown to cause myocardial dysfunction (29). In addition, peroxynitrite can activate MMP-2 which can cause myocardial dysfunction (30) by degrading troponin I (31) and myosin light chain (32). In newborn piglets subjected to hypoxia and reoxygenation, Borke et al found a significant increase in the gelatinolytic activity of MMP-2 of the left ventricle which was associated with a decreased antioxidant capacity (33). More recently, Haase et al demonstrated similar findings regarding the left ventricular MMP-2 activity (27). They also observed a significant positive correlation between left ventricular glutathione (intracellular antioxidant) levels, cardiac output and stroke volume.

Transient myocardial ischemia in neonatal asphyxia is underestimated in the literature (34). Thus, Barberi et al suggested that it would be useful to measure creatine phosphokinase-myocardial bound isoenzyme activity in all neonates suspected to have asphyxia. Patients identified with myocardial ischemia could then be submitted to an electrocardiogram for detection in order to offer opportune treatment (35). Gunes at al, in a study of 45 infants with different degrees of asphyxia, found a significant increase in troponin-T level in the severely asphyxiated infants which persisted for 7 days. The creatine phosphokinase-myocardial bound levels were high only at the age of 2-4h (36).

They also found significant echocardiographic changes in the group of severely asphyxiated neonates on day one in the form of tricuspid and mitral regurgitation and patent ductus arteriosus. They concluded that asphyxia-related cardiac involvements are significant and troponin T is a good determinant and more reliable in determining the cardiac involvement than other cardiac enzymes. Electrocardiography has been used in some reports as a tool to assess cardiac involvement in neonatal asphyxia. Electrocardiographic changes can vary from normal to acute myocardial infarction (37). ST-segment depression, indicating ventricular strain or ischemia, was significantly higher in asphyxiated neonates than controls (38). However, the electrocardiographic changes did not predict the outcome of asphyxiated neonates (38).

As a result of myocardial injury and dysfunction, signs of congestive heart failure, low cardiac output and primary pulmonary hypertension of the newborn will develop (39). Congestive heart failure is manifested as pulmonary congestion leading to respiratory distress and systemic congestion with hepatomegaly, edema and high central venous pressure. The clinical features of low cardiac output include hypotension, weak peripheral pulses, prolonged capillary refill time, oliguria and lactic acidosis (39). Primary pulmonary hypertension can result in severe hypoxia due to right-to-left shunting of deoxygenated blood through the ductus arteriosus and foramen ovale. The signs of cardiac involvement in neonatal asphyxia can mimic diseases such as hyaline membrane disease, transient tachypnea of the newborn, meconium aspiration syndrome and septic shock that may occur simultaneously with myocardial dysfunction (40). Therefore, it is very important to recognize each entity and treat accordingly (41).

Effects of hypoxia-reoxygenation on systemic, pulmonary and regional vasculatures

Hypoxia has a differential effect on the resistance of different vascular beds. It leads to an increase in pulmonary vascular resistance and pulmonary hypertension (42-45). On the other hand, the effect of hypoxia on systemic vascular resistance is variable. The inconsistency regarding the systemic vascular responses can be explained by differences in the duration and severity of hypoxia, animal species, vascular size, and experimental conditions (46,47).

In a newborn piglet model of hypoxia-reoxygenation, Cheung et al found that hypoxia induced systemic vasodilatation was followed by systemic hypotension (48). On the other hand, severe hypoxia can cause systemic vasoconstriction as well as pulmonary vasoconstriction and pulmonary hypertension (49). This could be due to decreased nitric oxide (50) and increased endothetlin-1 production (42). With reoxygenation, both pulmonary vascular resistance and pulmonary arterial pressure decreased. Systemic arterial pressure may not recover and, in fact, pressure decreases in spite of reoxygenation; this may be explained by myocardial stunning as well as decreased vascular resistance (48).

Cerebral blood flow is usually maintained (51)or increased in the initial phase of hypoxia but decreases if the hypoxia persists (52), whereas portal vein, mesenteric, hepatic (48) and renal blood flows (53) all decrease. During the early phase of reoxygenation, cerebral blood flow may increase, and correlate significantly with the PaO_2 (51). Similarly, mesenteric blood flow may increase during the initial phase of reoxygenation, it will then decrease or return back to normal depending on the severity of the hypoxia and reoxygenation injury (48,53). On the other hand, renal blood flow usually has a delayed recovery response to reoxygenation and may not recover if the insult is too severe (55,56).

During hypoxia, systemic oxygen delivery decreases, (48) which is associated with unchanged or decreased systemic oxygen consumption. The systemic oxygen extraction ratio increases. With the improvement in cardiac output following the initial phase of reoxygenation, systemic oxygen delivery may recover, but may subsequently deteriorate. These changes could be due to the changes in the cardiac output and the use of 100% oxygen in some animal models (27). Regional oxygen deliveries are decreased during hypoxia which may at least partially recover with reoxygenation (48,51,54,55).

Platelets pathology

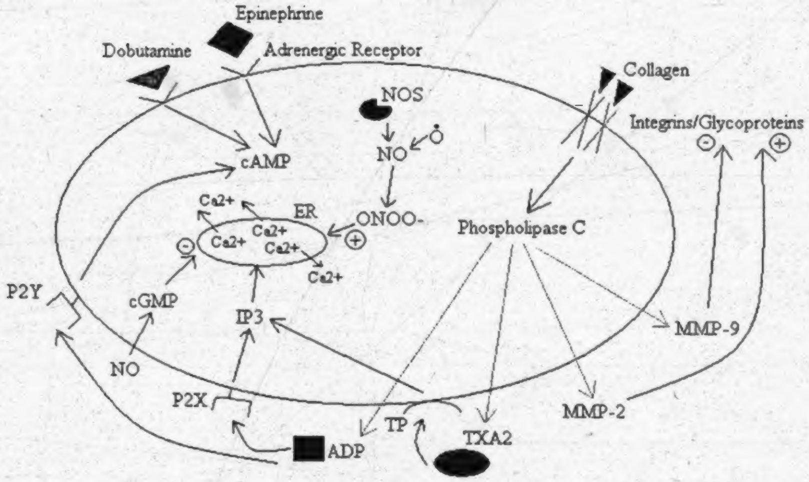
Platelet pathology can be manifest as changes in number and/or the function. It is essential to have normal platelet count and normal function in order to regulate the hemostatic balance. Thrombocytopenia ($<150 \times 10^9$/L) can lead to bleeding while thrombocytosis ($>450 \times 10^9$/L) can increase the risk for thrombosis. Platelet function abnormalities can also lead to similar effects. Thrombocytopenia can be caused by increased platelet destruction (sepsis, intravascular device, malaria, autoimmune diseases) (57) or decreased production (sepsis, drugs, neoplasms). Neonatal platelet dysfunction could be secondary to inherited (Glanzmann Thrombasthenia and Bernard-Soulier syndrome) or acquired disorders including prematurity, drugs (non-steroidal anti-inflammatory drugs), sepsis and asphyxia (58).

It has been shown that asphyxiated neonates have evidence of disseminated intravascular coagulation (59,60), low platelet count and high thromboxane release (61). In a study by Kaapa et al, they found decreased thromboxane B_2 formation in seven neonates with severe birth asphyxia (62). Platelets might be also involved in the mechanism of hypoxic-ischemic encephalopathy. Platelet-activating factor has been suggested to be involved in the neuronal damage in hypoxic-ischemic encephalopathy (63). Akisu et al showed a significant correlation between the severity of hypoxic-ischemic encephalopathy and the plasma concentration of platelet activating factor, but no correlation with platelet and leukocyte counts (63).

Effect of hypoxia-reoxygenation on platelets

Beside asphyxia (63), hypoxia-reoxygenation can lead to platelet activation and dysfunction (58,64). Hypoxia can result in inhibition of platelet aggregation *in vitro* (65) and shortening of platelet survival *ex vivo* (66). Reoxygenation after hypoxia is also accompanied by platelet dysfunction. Recently, Cheung et al found that the use of 100% oxygen for the resuscitation of newborn piglets is associated with platelet activation and platelet aggregatory dysfunction (58). In addition, there were significant increases in the plasma of TxB_2 and MMP-9 levels. The authors speculated that the use of 100% oxygen for reoxygenation causes the activation and release of MMP-9 and may aggravate the prostaglandin-thromboxane mechanistic pathway of platelet aggregation. In addition to the activation of MMPs (67), oxidative stress (superoxide anion and hydroxyl radical) can lead to platelet dysfunction (68,69). Furthermore, reactive nitrogen species such as peroxynitrite anion, known to activate MMPs (70), can cause platelet activation and aggregatory dysfunction (71).

Figure 2-1: Platelet activation and inhibitory pathways.


Epinephrine
Dobutamine
Adrenergic Receptor
Collagen
NOS
Integrins/Glycoproteins
⊖
⊕
cAMP
NO
Ȯ
ONOO-
⊕
Ca2+
ER
Ca2+
Ca2+
Ca2+
Ca2+
⊖
Phospholipase C
P2Y
cGMP
IP3
MMP-9
NO
P2X
TP
MMP-2
TXA2
ADP

ADP: adenosine diphosphate; cAMP: cyclic 3'-5'-adenosine monophosphate; cGMP: cyclic 3'-5'-guanosine monophosphate, ER: endoplasmic reticulum; IP_3: inositol 1,4,5-trisphosphate; MMP: matrix metalloproteinase; NO: nitric oxide; NOS: nitric oxide synthase; $ONOO^-$: peroxynitrite; TxA_2: thromboxane A_2.

References

1. Lee J, Rajadurai VS, Tan KW 1999 Blood pressure standards for very low birthweight infants during the first day of life. Arch Dis Child Fetal Neonatal Ed 81:F168-F170
2. Nuntnarumit P, Yang W, Bada-Ellzey HS 1999 Blood pressure measurements in the newborn. Clin Perinatol 26:981-996
3. Kluckow M, Evans N 1996 Relationship between blood pressure and cardiac output in preterm infants requiring mechanical ventilation. J Pediatr 129:506-512
4. Kluckow M, Evans N 2000 Superior vena cava flow in newborn infants: a novel marker of systemic blood flow. Arch Dis Child Fetal Neonatal Ed 82:F182-F187
5. Tsuji M, Saul JP, du PA, Eichenwald E, Sobh J, Crocker R, Volpe JJ 2000 Cerebral intravascular oxygenation correlates with mean arterial pressure in critically ill premature infants. Pediatrics 106:625-632
6. Guyton AC 2006 Hemostasis and blood coagulation In: Guyton AC (ed) Textbook of medical physiology. Elsevier Saunders, Philadelphia, pp 457-467
7. Clemetson KJ 2002 Platelets receptors. In: Michelson AD (ed) Platelets. Academic Press, San Diego, CA, pp 65-84
8. Woulfe D, Yang J, Prevost N, O'Brien PJ, Brass LF 2002 Signal transdution during the initiation, extension and perpetuation of platelet plug formation. In: Michelson AD (ed) Platelets. Academic Press, San Diego, CA, pp 197-213
9. Schwarz UR, Walter U, Eigenthaler M 2001 Taming platelets with cyclic nucleotides. Biochem Pharmacol 62:1153-1161
10. Hooper NM 1994 Families of zinc metalloproteases. FEBS Lett 354:1-6

11. Sawicki G, Salas E, Murat J, Miszta-Lane H, Radomski MW 1997 Release of gelatinase A during platelet activation mediates aggregation. Nature 386:616-619

12. Fernandez-Patron C, Martinez-Cuesta MA, Salas E, Sawicki G, Wozniak M, Radomski MW, Davidge ST 1999 Differential regulation of platelet aggregation by matrix metalloproteinases-9 and -2. Thromb Haemost 82:1730-1735

13. Lee YM, Lee JJ, Shen MY, Hsiao G, Sheu JR 2006 Inhibitory mechanisms of activated matrix metalloproteinase-9 on platelet activation. Eur J Pharmacol 537:52-58

14. Yabek SM, Kato R, Singh BN 1985 Effects of hypoxia on the cellular electrical activity of adult and neonatal canine ventricular myocardium. Pediatr Res 19:1263-1267

15. Anderson SE, Liu H, Ho HS, Lewis EJ, Cala PM 2003 Age-related differences in Na+-dependent Ca2+ accumulation in rabbit hearts exposed to hypoxia and acidification. Am J Physiol 284:C1123-C1132

16. Jarmakani JM, Nakazawa M, Nagatomo T, Langer GA 1978 Effect of hypoxia on mechanical function in the neonatal mammalian heart. Am J Physiol 235:469-474

17. Su JY, Higgins CB, Friedman WF, Nyhan WL 1973 Chronotropic and inotropic effects of prostaglandins E1, A1, and F2alpha on isolated mammalian cardiac tissue. Proc Soc Exp Biol Med 143:1227-1230

18. Penney DG, Cascarano J 1970 Anaerobic rat heart. Effects of glucose and tricarboxylic acid-cycle metabolites on metabolism and physiological performance. Biochem J 118:221-227

19. Jones CT, Rolph TP 1985 Metabolism during fetal life: a functional assessment of metabolic development. Physiol Rev 65:357-430

20. Downing SE, Talner NS, Gardner TH 1966 Influences of hypoxemia and acidemia on left ventricular function. Am J Physiol 210:1327-1334

21. Talner NS, Gardner TH, Downing SE 1966 Influence of acidemia on left ventricular function in the newborn lamb. Pediatrics 38:457-464

22. Downing SE, Talner NS, Gardner TH 1966 Influences of arterial oxygen tension and pH on cardiac function in the newborn lamb. Am J Physiol 211:1203-1208

23. Katz AM, Hecht HH 1969 Editorial: the early "pump" failure of the ischemic heart. Am J Med 47:497-502

24. Nakanishi T, Okuda H, Nakazawa M, Takao A 1985 Effect of acidosis on contractile function in the newborn rabbit heart. Pediatr Res 19:482-488

25. Yee ES, Ebert PA 1979 Effect of ischemia on ventricular function, compliance, and edema in immature and adult canine hearts. Surg Forum 30:250-252

26. Kohman LJ Veit LJ 1991 Neonatal myocardium resists reperfusion injury. J Surg Res. 51:133-7

27. Haase E, Bigam DL, Nakonechny QB, Rayner D, Korbutt G, Cheung PY 2005 Cardiac function, myocardial glutathione, and matrix metalloproteinase-2 levels in hypoxic newborn pigs reoxygenated by 21%, 50%, or 100% oxygen. Shock 23:383-389

28. Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB 1988 Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest 82:476-485

29. Yasmin W, Strynadka KD, Schulz R 1997 Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. Cardiovasc Res 33:422–432

30. Cheung PY, Sawicki G, Wozniak M, Wang W, Radomski MW, Schulz R 2000 Matrix metalloproteinase-2 contributes to ischemia-reperfusion injury in the heart. Circulation 101:1833-1839

31. Wang W, Schulze CJ, Suarez-Pinzon WL, Dyck JRB, Sawicki G, Schulz R 2002 Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. Circulation 106:1543-1549

32. Sawicki G, Leon H, Sawicka J, Sariahmetoglu M, Schulze CJ, Scott PG, Szczesna-Cordary D, Schulz R 2005 Degradation of myosin light chain in isolated at hearts subjected to ischemia-reperfusion injury: A New Intracellular Target for Matrix Metalloproteinase-2. Circulation 112:544-552

33. Borke WB, Munkeby BH, Halvorsen B, Bjornland K, Tunheim SH, Borge GI, Thaulow E, Saugstad OD 2004 Increased myocardial matrix metalloproteinases in hypoxic newborn pigs during resuscitation: effects of oxygen and carbon dioxide. Eur J Clin Invest 34:459-466

34. Tapia-Rombo CA, Carpio-Hernandez JC, Salazar-Acuna AH, varez-Vazquez E, Mendoza-Zanella RM, Perez-Olea V, Rosas-Fernandez C 2000 Detection of transitory myocardial ischemia secondary to perinatal asphyxia. Arch Med Res 31:377-383

35. Barberi I, Calabro MP, Cordaro S, Gitto E, Sottile A, Prudente D, Bertuccio G, Consolo S 1999 Myocardial ischaemia in neonates with perinatal asphyxia.

Electrocardiographic, echocardiographic and enzymatic correlations. Eur J Pediatr 158:742-747

36. Gunes T, Ozturk MA, Koklu SM, Narin N, Koklu E 2005 Troponin-T levels in perinatally asphyxiated infants during the first 15 days of life. Acta Paediatr 94:1638-1643

37. Gidvani CH, Raju U, Chandar V, Ghosh B, Wilson CG 1990 ECG changes in asphyxia neonatorum. Indian Pediatr 27:1177-1181

38. Omokhodion SI, Jaiyesimi F, Losekoot TG 2003 Standard 12 lead and 24 hour Holter electrocardiographic observation in a biracial group of perinatally asphyxiated newborns. West Afr J Med 22:253-258

39. Ranjit MS 2000 Cardiac abnormalities in birth asphyxia. Indian J Pediatr 67:S26-S29

40. Greenough A, Khetriwal B 2005 Pulmonary hypertension in the newborn. Paediatr Respir Rev 6:111-6

41. Piazza AJ 1999 Postasphyxial management of the newborn. Clin Perinatol 26:749-65

42. Medbo S, Yu XQ, Asberg A, Saugstad OD 1998 Pulmonary hemodynamics and plasma endothelin-1 during hypoxemia and reoxygenation with room air or 100% oxygen in a piglet model. Pediatr Res 44:843-849

43. Rodman DM, Yamaguchi T, O'Brien RF, McMurtry IF 1989 Hypoxic contraction of isolated rat pulmonary artery. J Pharmacol Exp Ther 248:952-959

44. Demiryurek AT, Wadsworth RM, Kane KA 1991 Effects of hypoxia on isolated intrapulmonary arteries from the sheep. Pulm Pharmacol 4:158-164

45. Leach RM, Twort CH, Cameron IR, Ward JP 1992 A comparison of the pharmacological and mechanical properties in vitro of large and small pulmonary arteries of the rat. Clin Sci (Lond) 82:55-62

46. Madden JA, Dawson CA, Harder DR 1985 Hypoxia-Induced Activation in Small Isolated Pulmonary-Arteries from the Cat. J of Appl Physiol 59:113-118

47. Madden JA, Vadula MS, Kurup VP 1992 Effects of Hypoxia and other vasoactive agents on pulmonary and cerebral-artery smooth-muscle cells. Am J of Physiol 263:L384-L393

48. Cheung PY, Barrington KJ, Bigam DL 1998 Temporal effects of prolonged hypoxaemia and reoxygenation on systemic, pulmonary and mesenteric perfusions in newborn piglets. Cardiovasc Res 39:451-458

49. Vanhoutte PM, Luscher TF, Graser T 1991 Endothelium-dependent contractions. Blood Vessels 28:74-83

50. Moore P, Velvis H, Fineman JR, Soifer SJ, Heymann MA 1992 EDRF inhibition attenuates the increase in pulmonary blood flow due to oxygen ventilation in fetal lambs. J Appl Physiol 73:2151-2157

51. Richards JG, Todd KG, Emara M, Haase E, Cooper SL, Bigam DL, Cheung PY 2006 A dose-response study of graded reoxygenation on the carotid haemodynamics, matrix metalloproteinase-2 activities and amino acid concentrations in the brain of asphyxiated newborn piglets. Resuscitation 69:319-327

52. Bauer R, Bergmann R, Walter B, Brust P, Zwiener U, Johannsen B 1999 Regional distribution of cerebral blood volume and cerebral blood flow in newborn piglets--effect of hypoxia/hypercapnia. Brain Res Dev Brain Res 112:89-98

53. Gouyon JB, Vallotton M, Guignard JP 1987 The newborn rabbit: a model for studying hypoxemia-induced renal changes. Biol Neonate 52:115-120

54. Haase E, Bigam DL, Nakonechny QB, Jewell LD, Korbutt G, Cheung PY 2004 Resuscitation with 100% oxygen causes intestinal glutathione oxidation and reoxygenation injury in asphyxiated newborn piglets. Ann Surg 240:364-373

55. Rootwelt T, Odden JP, Hall C, Saugstad OD 1996 Regional blood flow during severe hypoxemia and resuscitation with 21% or 100% O2 in newborn pigs. J Perinat Med 24:227-236

56. Haase E. Graded reoxygenation of asphyxiated neonates. MSc Thesis. 2003. University of Alberta. Thesis/Dissertation

57. Castle V, Andrew M, Kelton J, Giron D, Johnston M, Carter C 1986 Frequency and mechanism of neonatal thrombocytopenia. J Pediatr 108:749-755

58. Cheung PY, Stevens JP, Haase E, Stang L, Bigam DL, Etches W, Radomski MW 2006 Platelet dysfunction in asphyxiated newborn piglets resuscitated with 21% and 100% oxygen. Pediatr Res 59:636-640

59. Suzuki S, Morishita S 1998 Hypercoagulability and DIC in high-risk infants. Semin Thromb Hemost 24:463-466

60. Fichera A, Pratico G, Sciacca F 1989 Neonatal hypoxia and hemocoagulative changes. Pediatr Med Chir 11:643-647

61. Brus F, van OW, Okken A, Oetomo SB 1997 Number and activation of circulating polymorphonuclear leukocytes and platelets are associated with neonatal respiratory distress syndrome severity. Pediatrics 99:672-680

62. Kaapa P, Viinikka L, Ylikorkala O 1984 Thromboxane-B_2 production by fetal and neonatal platelets: effect of idiopathic respiratory-distress syndrome and birth asphyxia. Pediatr Res 18:756-758

63. Akisu M, Kultursay N, Coker I, Huseyinov A 1998 Plasma platelet-activating factor levels in newborn infants with and without perinatal asphyxia: Is it an additional marker of perinatal asphyxia? Acta Paediatr Jpn 40:427-431

64. Leo R, Pratico D, Iuliano L, Pulcinelli FM, Ghiselli A, Pignatelli P, Colavita AR, FitzGerald GA, Violi F 1997 Platelet activation by superoxide anion and hydroxyl radicals intrinsically generated by platelets that had undergone anoxia and then reoxygenated. Circulation 95:885–891

65. Akahori M, Uedono Y, Yamagami K, Takeyama N, Kitazawa Y, Tanaka T 1995 Hypoxia alters the energy metabolism and aggregation of washed human platelets. Haematologia (Budap) 26:191–198

66. Castle V, Coates G, Mitchell LG, O'Brodovich H, Andrew M 1988 The effect of hypoxia on platelet survival and site of sequestration in the newborn rabbit. Thromb Haemost 59:45-48

67. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS 1996 Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 98: 2572–2579

68. Krotz F, Sohn HY, Pohl U 2004 Reactive oxygen species: players in the platelet game. Arterioscler Thromb Vasc Biol 24:1988-1996

69. Leo R, Pratico D, Iuliano L, Pulcinelli FM, Ghiselli A, Pignatelli P, Colavita AR, FitzGerald GA, Violi F 1997 Platelet activation by superoxide anion and hydroxyl radicals intrinsically generated by platelets that had undergone anoxia and then reoxygenated. Circulation 95:885-891

70. Okamoto T, Akaike T, Nagano T, Miyajima S, Suga M, Ando M, Ichimori K, Maeda H 1997 Activation of human neutrophil procollagenase by nitrogen dioxide and peroxynitrite: a novel mechanism for procollagenase activation involving nitric oxide. Arch Biochem Biophys 342:261-274

71. Brown AS, Moro MA, Masse JM, Cramer EM, Radomski M, Darley-Usmar V 1998 Nitric oxide-dependent and independent effects on human platelets treated with peroxynitrite. Cardiovasc Res 40:380-388

CHAPTER 3

Hemodynamic support after neonatal asphyxia

Management of neonatal asphyxia

After the initial resuscitation of asphyxiated neonates, management strategies depend on the extent of organ dysfunction and mainly involve symptomatic support. Respiratory support ranges from the administration of oxygen, mechanical ventilation to inhaled nitric oxide for the treatment of respiratory failure and pulmonary hypertension. The aim is to maintain normal blood gases and acid-base balance. Acidosis may worsen cardiac function (1). Hypercapnia may lead to systemic vasodilatation and pulmonary vasoconstriction (2), therefore, aggravating systemic hypotension and pulmonary hypertension, respectively. Hypocapnia however can cause cerebral vasoconstriction and worsen cerebral ischemia (3).

Cardiovascular support for hypotension and myocardial dysfunction includes fluid and/or inotropic support. The aim is to restore normal tissue perfusion. Such intervention is crucial, hypotension may aggravate tissue ischemia and hypertension may lead to intracranial hemorrhage. Gastrointestinal support varies from delayed feeding and parenteral nutrition in case of suspected intestinal ischemia to surgical intervention if gastrointestinal perforation occurs. Renal support requires meticulous fluid and electrolyte balance. Fluid overload can contribute to cerebral edema and cardiopulmonary failure. Dehydration can lead to hypotension and compromises tissue perfusion. Hematological support includes blood products transfusion to correct anemia, thrombocytopenia and disseminated intravascular coagulopathy. Antibiotics are often administered to treat accompanying or underlying infection. Correcting metabolic derangements such as hypoglycemia, hypocalcemia and hyponatremia is also important to prevent further damage to the brain and other systems. The support to central nervous system may include antiseizure medications and more recently, head cooling or total body cooling (induced hypothermia) (4,5). The latter is showing promising results but still considered experimental and not the standard of care (6).

Finally, it is very important to mention that treating one particular organ dysfunction can have a variable impact on another organ. For example, the administration of fluid to correct hypotension in asphyxiated neonates may worsen pulmonary and cerebral edema although there are controversies over the use of colloid vs. normal saline in fluid to be administered. Therefore, it is prudent to choose the appropriate therapy that has minimum systemic and regional side effects.

Hemodynamic support in neonatal asphyxia

Skouteli et al found that hypotension in neonates to be a poor predictor for survival and normal neurodevelopmental outcomes (7). However, Kluckow and Evans showed that the superior vena caval flow was a better predictor of short term morbidities and neurodevelopmental outcome at early childhood (8,9). It is understandable that there is a weak but significant correlation between systemic blood pressure and superior vena caval flow (a measure of cardiac output) (10). Although systemic arterial pressure and cardiac output are related (flow = pressure ÷ resistance), they are not the same. A number of factors may affect this relationship including myocardial function, shunting of blood from the systemic to pulmonary circulations through a patent ductus arteriosus or foramen ovale (11,12), varying pulmonary and peripheral vascular resistance, and the use of positive-pressure ventilation (13). Therefore, the concept of "permissive hypotension", with the therapies directed to situations with pathologically low systemic arterial pressure, has been suggested (14). Therefore, the treatment of neonatal myocardial dysfunction and hypotension should be directed to the underlying cause aiming to restore normal tissue perfusion. In general, neonatal hypotension following asphyxia can be due to pump failure (myocardial dysfunction), a decrease in venous return (hypovolemia) and vasodilatation (reoxygenation injury) (15). The treatment may include fluid boluses, vasopressors or inotropes and steroids.

Fluid Therapy

Giving fluid is crucial to a neonate who is volume-depleted. On the other hand, the administration of fluid to a hypotensive neonate who has myocardial dysfunction following asphyxia can worsen heart failure and the general condition. Instead, neonates with heart failure need to be fluid restricted. It is also important to note that neonatal asphyxia is associated with cerebral edema and fluid overload might also worsen this condition. However, in neonates with asphyxia secondary to hemorrhage or sepsis, giving fluid and blood products are essential for proper hemodynamic responses towards inotropes. Therefore, fluid therapy should be given with meticulous attention to the intravascular fluid balance (16).

The current evidence does not support the routine use of volume expansion to improve the clinical outcome (17). The use of volume expansion can lead to an increase in left ventricular output and superior vena caval flow (18,19). However, it is not known if these responses are transient since the measurements were taken immediately after volume expansion. Finally, volume expansion was found to be less effective than dopamine in improving blood pressure (20) and there is a lack of evidence to support volume depletion in hypotensive asphyxiated neonates.

Inotropes

Theoretically, an ideal inotrope is a drug that has a positive inotropic action and an after-load reduction effect to enhance myocardial function and systemic oxygen delivery as well as to minimize the work load caused by the increased peripheral vascular resistance. Interestingly, the after-load reduction effect may not be beneficial or indeed be harmful in asphyxiated neonates who have a reduced systemic vascular resistance following reoxygenation (21). It is also important for the drug to have minimum side effects on the systemic and myocardial oxygen consumption. In addition, particularly in asphyxiated neonates, an ideal inotrope should have no aggravating effect on the pulmonary vascular resistance. If an inotrope causes imbalance between the pulmonary and systemic vascular resistance, shunting across the ductus arteriosus or foramen ovale may occur in the direction of the vascular bed with low resistance. Asphyxia is associated with primary pulmonary hypertension of the newborn which may result in severe respiratory failure (22). The Clinician must be careful in choosing an inotrope to treat the accompanying hypotension but not to worsen the pulmonary hypertension by increasing the pulmonary vascular resistance.

Dopamine

Dopamine is an agonist for dopaminergic and α and β adrenergic receptors. Dopamine is a useful vasopressor to increase the blood pressure in hypotensive newborns (19). It operates at a high dose (10 μg/kg/min) to achieve β-adrenergic agonism, which is opposed by the concurrent α-adrenergic stimulation. While Lundstrom et al demonstrated an increase in left ventricular output in premature newborns (19), Roze et al interestingly showed that dopamine did not increase cardiac output, and indeed cardiac output appeared to decrease in certain patients (23). High dose dopamine infusion had a detrimental effect on the ratio of systemic to pulmonary pressures[i] with no significant effect on cardiac index (24,25). Moreover, the findings did not support the use of dopamine to improve renal and mesenteric perfusion after asphyxia as also evidenced in a meta-analysis of its use in critically ill newborns (26). Although Seri et al have shown that low-dose dopamine infusion increases urine output in non-hypotensive preterm newborns (27), the observed clinical effect may be related to an α-adrenoceptor mediated vasopressor effect (increasing blood pressure) instead of a true renal dopaminergic effect (28).

In systematic reviews, dopamine has been found to be superior to fluid boluses (20) and dobutamine infusion (117) in improving systemic blood pressure. However there were no differences in heart rate and the short term morbidity (intraventricular hemorrhage, periventricular leukomalacia) or mortality between dopamine and dobutamine treated patients.

There is very limited data that examined the effect of dopamine in term infants with perinatal asphyxia. Administration of dopamine at low doses to prevent morbidities and mortality associated perinatal asphyxia did not result in any difference between treatment and control groups (29).

---

[i] Ductus arteriosus usually remains patent in sick or hypoxic newborns. The ratio of pulmonary to systemic pressures is critically important for the direction of shunting across the ductus arteriosus, which determines the oxygen content of the blood distributed to various organs. Systemic oxygen saturation falls as a consequence of a high ratio.

Epinephrine

Epinephrine is effective in treating hypotension as well as increasing cardiac output because of its α and β-adrenergic stimulation. However, epinephrine is often reserved as the "rescue" inotrope due to its peripheral vasoconstriction, particularly in the mesenteric and renal vascular beds. Interestingly, a blunted vasoconstrictive effect of epinephrine in the regional circulation by hypoxia has been found, which may be via a reduction in α mediated vasoconstriction (30) and/or increased β-adrenoceptors responsiveness (31). It was previously reported that positive inotropic effect of epinephrine, with improved mesenteric and renal perfusion and no significant vasoconstriction, was observed during normoxia and hypoxia (32,33) and reoxygenation after asphyxia (34). Although epinephrine causes an increase in the pulmonary blood pressure, this is associated with increased systemic arterial pressure resulting in favorable pulmonary/systemic pressure ratio. Because of its inotropic and vasopressor actions, epinephrine is an effective agent to increase the systemic arterial pressure during reoxygenation in asphyxiated newborns. However, high dose of epinephrine can increase pulmonary vascular resistance (32) which is a known complication of neonatal asphyxia. It can also lead to systemic vasoconstriction and impede tissue perfusion which may increase lactate production (35).

Dobutamine

Dobutamine has potent β-adrenoceptor effects that are accompanied by some α effects. The d and l isoforms have varying degrees of agonistic activity at α and β receptors (36). It seems that low dose dobutamine infusion may be an effective inotrope in the treatment of normotensive shock in newborns with without any significant pulmonary arterial pressure (37). However, dobutamine is not effective in normalizing low blood pressure. Dobutamine may behave like a double-edged sword with vasodilatation at low doses and vasoconstriction at high dose because of controversial findings at regional flows (38). Although Crowley et al showed an 11% decrease in pulmonary arterial pressure with dobutamine at 5-10 µg/kg/min (37), dobutamine may cause an unfavorable effect on the pulmonary to systemic pressure ratio, which reverses the ductal shunt and offsets any increase in oxygen delivery (39). Moreover, at doses of 20 µg/kg/min or greater, dobutamine caused significant pulmonary hypertension and possible mesenteric (40) and renal vasoconstriction (39). Despite of the reports on premature neonates with hypotension and normoxic and hypoxic animal models, little information is available on the effects of dobutamine in hypotension and myocardial stunning following the reoxygenation of asphyxiated neonates.

Steroids

Among various organs, neonatal asphyxia may involve the adrenal glands. The administration of steroids may seem like a reasonable option (41). Brief steroid treatment given to neonates with systemic hypotension stabilizes the cardiovascular status and decreases the need for inotropes (42). The mechanism by which steroid treatment improves the cardiovascular status could be related to an up-regulation of adrenergic receptors on the vascular walls and the myocardium (43). Giving steroids, particularly in infants, deserves further examination because of the association between postnatal steroids treatment and poor neurodevelopmental outcome (44,45). There are no data to either support or discourage the use of steroids in the setting of hypotension secondary to neonatal asphyxia. Therefore, more research is needed in this area before recommendations can be made.

References

1. Talner NS, Gardner TH, Downing SE 1966 Influence of acidemia on left ventricular function in the newborn lamb. Pediatrics 38:457-464
2. Lee KJ, Hernandez G, Gordon JB 2003 Hypercapnic acidosis and compensated hypercapnia in control and pulmonary hypertensive piglets. Pediatr Pulmonol 36:94-101
3. Rosenberg AA 1992 Response of the cerebral circulation to hypocarbia in post-asphyxia newborn lambs. Pediatr Res 32:537-541
4. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD, Finer NN, Carlo WA, Duara S, Oh W, Cotten CM, Stevenson DK, Stoll BJ, Lemons JA, Guillet R, Jobe AH 2005 Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med 353:1574-1584
5. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P 2005 Cooling for newborns with hypoxic ischaemic encephalopathy. Pediatr Res 58:385
6. Gunn AJ, Thoresen M 2006 Hypothermic neuroprotection. NeuroRx 3:154-169
7. Skouteli HN, Dubowitz LM, Levene MI, Miller G 1985 Predictors for survival and normal neurodevelopmental outcome of infants weighing less than 1001 grams at birth. Dev Med Child Neurol 27:588-595
8. Kluckow M, Evans N 2000 Low superior vena cava flow and intraventricular haemorrhage in preterm infants. Arch Dis Child Fetal Neonatal Ed 82:F188-F194
9. Hunt RW, Evans N, Rieger I, Kluckow M 2004 Low superior vena cava flow and neurodevelopment at 3 years in very preterm infants. J Pediatr 145:588-592

10. Kluckow M, Evans N 1996 Relationship between blood pressure and cardiac output in preterm infants requiring mechanical ventilation. J Pediatr 129:506-512

11. Evans N, Iyer P 1994 Assessment of ductus arteriosus shunt in preterm infants supported by mechanical ventilation: effects of interatrial shunting. J Pediatr 125:778–785.

12. Evans N Iyer P 1994 Incompetence of the foramen ovale in preterm infants supported by mechanical ventilation. J Pediatr 125:786–792.

13. Evans N, Kluckow M 1996 Early determinants of right and left ventricular output in ventilated preterm infants. Arch Dis Child Fetal Neonatal Ed 74:F88-94

14. Dempsey E M., Alhazzani F, Barrington K 2005 Permissive Hypotension in the extreme low birth weight. Pediatr Res 57: 560 Abstract

15. Haase E, Bigam DL, Nakonechny QB, Rayner D, Korbutt G, Cheung PY 2005 Cardiac function, myocardial glutathione, and matrix metalloproteinase-2 levels in hypoxic newborn pigs reoxygenated by 21%, 50%, or 100% oxygen. Shock 23:383-389

16. Piazza AJ 1999 Postasphyxial management of the newborn. Clin Perinatol 26:749-65

17. Subhedar NV, Shaw NJ 2003 Dopamine versus dobutamine for hypotensive preterm infants. Cochrane Database Syst RevCD001242

18. Lundstrom K, Pryds O, Greisen G 2000 The haemodynamic effects of dopamine and volume expansion in sick preterm infants. Early Hum Dev 57:157-163

19. Pladys P, Wodey E, Betremieux P, Beuchee A, Ecoffey C 1997 Effects of volume expansion on cardiac output in the preterm infant. Acta Paediatr 86:1241-1245

20. Osborn DA, Evans N 2001 Early volume expansion versus inotrope for prevention of morbidity and mortality in very preterm infants. Cochrane Database Syst Rev CD002056

21. Cheung PY, Barrington KJ, Bigam DL 1998 Temporal effects of prolonged hypoxaemia and reoxygenation on systemic, pulmonary and mesenteric perfusions in newborn piglets. Cardiovasc Res 39:451-458

22. Hsieh WS, Yang PH, Fu RH 2001 Persistent pulmonary hypertension of the newborn: experience in a single institution. Acta Paediatr Taiwan 42:94-100

23. Roze JC, Tohier C, Maingueneau C, Lefevre M, Mouzard A 1993 Response to dobutamine and dopamine in the hypotensive very preterm infant. Arch Dis Child 69:59-63

24. Mentzer RM, Jr., Alegre C, Nolan SP 1976 Effect of dopamine and isoproterenol on pulmonary vascular resistance. Rev Surg 33:433-436

25. Barrington KJ, Finer NN, Chan WK 1995 A blind, randomized comparison of the circulatory effects of dopamine and epinephrine infusions in the newborn piglet during normoxia and hypoxia. Crit Care Med 23:740-748

26. Prins I, Plotz FB, Uiterwaal CSPM, van Vught HJ 2001 Low-dose dopamine in neonatal and pediatric intensive care: a systematic review. Intensive Care Medicine 27:206-210

27. Seri I, Abbasi S, Wood DC, Gerdes JS 1998 Regional hemodynamic effects of dopamine in the sick preterm neonate. Journal of Pediatrics 133:728-734

28. Nguyen LB, Lievano G, Radhakrishnan J, Fornell LC, Jacobson G, John EG 1999 Renal effects of low to moderate doses of dopamine in newborn piglets. J Pediatr Surg 34:996-999

29. DiSessa TG, Leitner M, Ti CC, Gluck L, Coen R, Friedman WF 1981 The cardiovascular effects of dopamine in the severely asphyxiated neonate. J Pediatr 99:772-6.

30. Tateishi J, Faber JE 1995 Atp-Sensitive K+ Channels Mediate Alpha(2D)-Adrenergic Receptor Contraction of Arteriolar Smooth-Muscle and Reversal of Contraction by Hypoxia. Circ Res 76:53-63

31. Lock JE, Olley PM, Coceani F 1981 Enhanced beta-adrenergic-receptor responsiveness in hypoxic neonatal pulmonary circulation. Am J Physiol 240:H697-H703

32. Cheung PY, Barrington KJ, Pearson RJ, Bigam DL, Finer NN, Van Aerde JE 1997 Systemic, pulmonary and mesenteric perfusion and oxygenation effects of dopamine and epinephrine. Am J Respir Crit Care Med 155:32-37

33. Cheung PY, Barrington KJ 2001 The effects of dopamine and epinephrine on hemodynamics and oxygen metabolism in hypoxic anesthetized piglets. Crit Care 5:158-166

34. Obaid L, Johnson S, Bigam D, and Cheung P-Y 2005 Systemic and regional hemodynamic effects of dopamine and epinephrine after hypoxia-reoxygenation in newborn piglets. Pediatric Academic Societies' Annual Meeting: Abstract 57: 531

35. Valverde E, Pellicer A, Madero R, Elorza D, Quero J, Cabanas F 2006 Dopamine versus epinephrine for cardiovascular support in low birth weight infants: analysis of systemic effects and neonatal clinical outcomes. Pediatrics 117:e1213-e1222

36. Ruffolo RR, Jr., Messick K 1985 Effects of dopamine, (+/-)-dobutamine and the (+)- and (-)-enantiomers of dobutamine on cardiac function in pithed rats. J Pharmacol Exp Ther 235:558-565

37. Crowley MR, Fineman JR, Soifer SJ 1991 Effects of vasoactive drugs on thromboxane A2 mimetic-induced pulmonary hypertension in newborn lambs. Pediatr Res 29:167-172

38. Nudel DB, Peterson BJ, Buckley BJ, Gootman N, Hoffman RG 1991 Effects of Acute Hypoxemia on Responses to Dopamine and Dobutamine in Neonatal Swine. Methods Find in Exp and Clin Pharmacol 13:111-119

39. Cheung PY, Barrington KJ, Bigam D 1999 The hemodynamic effects of dobutamine infusion in the chronically instrumented newborn piglet. Crit Care Med 27:558-564

40. Ferrara JJ, Dyess DL, Peeples GL, Christenberry DP, Roberts WS, Tacchi EJ, Swafford AN, Ardell JL, Powell RW 1995 Effects of dopamine and dobutamine on regional blood flow distribution in the neonatal piglet. Ann Surg 221:531-540

41. Aucott SW 2005 Hypotension in the newborn: who needs hydrocortisone? J Perinatol 25:77-78

42. Ng PC, Lee CH, Bnur FL, Chan IH, Lee AW, Wong E, Chan HB, Lam CW, Lee BS, Fok TF 2006 A double-blind, randomized, controlled study of a "stress dose" of

hydrocortisone for rescue treatment of refractory hypotension in preterm infants. Pediatrics 117:367-375

43. Sasidharan P 1998 Role of corticosteroids in neonatal blood pressure homeostasis. Clin Perinatol 25:723-40

44. Stark AR, Carlo WA, Tyson JE, Papile LA, Wright LL, Shankaran S, Donovan EF, Oh W, Bauer CR, Saha S, Poole WK, Stoll BJ 2001 Adverse effects of early dexamethasone in extremely-low-birth-weight infants. N Engl J Med 344:95-101

45. Yeh TF, Lin YJ, Huang CC, Chen YJ, Lin CH, Lin HC, Hsieh WS, Lien YJ 1998 Early dexamethasone therapy in preterm infants: a follow-up study. Pediatrics 101:E7-E7

CHAPTER 4

Dobutamine

Chemistry

Dobutamine is a synthetic sympathomimetic agent that is structurally related to dopamine but possesses a bulky aromatic substituent on the amino group (Figure 4-1). It is generally considered a relatively selective β-adrenergic agonist (1,2). Dobutamine acts directly on the β-adrenoceptor. In contrast, dopamine acts through the release of norepinephrine (3). Dopamine therefore, has the disadvantage of limited response once the norepinephrine stores are depleted. Dobutamine has a center of asymmetry; both enantiomeric forms are present in the racemic mixture used clinically. The (-) isomer of dobutamine is a potent agonist at α_1-adrenoceptor and can result in marked vasoconstriction. On the other hand, (+)-dobutamine is a potent α_1-adrenoceptor antagonist, which can block the effects of (-)-dobutamine. The effects of these two isomers are mediated via β-adrenoceptor. The (+) isomer is a more potent (approximately tenfold) β-adrenoceptor agonist than the (-) isomer and the net result will be β-adrenergic stimulation (2).

Dobutamine occurs as a white to off-white, crystalline powder and is soluble in water and in alcohol. Dobutamine has a pH of 9.4. It is commercially available as a sterile solution as dobutamine hydrochloride ($C_{18}H_{23}NO_3.HCl$) with a pH of 2.5-5.5. It can be diluted with dextrose solution, normal saline or Ringer's lactate. The drug solution is colorless to faint straw-color. This coloration is due to minimum oxidation that does not affect the drug potency. It should not be diluted with other drugs and should not be mixed with sodium bicarbonate. It is stable at room temperature. Dobutamine hydrochloride may contain sulfite which is considered responsible for the allergic reaction that may occur during dobutamine infusion.

Pharmacokinetics

Dobutamine is administered intravenously (2). It acts very quickly with an onset of time of about 2 min. The action peaks at 10 min. It has a plasma half life of 2 min. It is metabolized in the liver by the enzyme catechol-O-methyltransferase and by glucuronidation to inactive metabolites and then excreted mainly by the kidneys.

Therapeutic uses

Dobutamine is a β-adrenoceptor agonist with some α-adrenoceptor effects. The drug can therefore increase cardiac output and blood pressure, presumably the effect on peripheral vascular resistance is not significant. In general dobutamine is used to treat acute cardiac decompensation following congestive heart failure, cardiac surgery, cardiomyopathies and acute myocardial infarction (4). It is considered as the standard inotropic agent in the treatment of septic shock (5). It is also used to treat systemic hypotension among all age groups (6). Moreover, dobutamine can be used for diagnostic purposes such as in dobutamine stress echocardiography which is used to predict cardiac events in patients with known or suspected coronary artery disease (7).

Cardiovascular effects of dobutamine in neonates

Dobutamine is commonly used in neonates with hypotension of any etiology, with the goal of improving cardiac output and preventing its detrimental consequences. Despite of its clinical use, there is little information on the effect of dobutamine in neonates with compromised cardiovascular state. There is no study identified, comparing dobutamine to other inotropic agents in term neonates with suspected asphyxia.

There are studies in the literature about the use of dobutamine in preterm neonates with hypotension. In a systematic review, dobutamine was found to be less effective than dopamine in normalizing systemic arterial pressure (8). In fact, dobutamine failed to improve systemic arterial pressure in about one third of the hypotensive preterm neonates (8). But, when other studies looked at blood flow as an end point rather than the systemic arterial pressure, dobutamine was found to be better in increasing blood flow through the superior vena cava, which implied a better cardiac output, than that of dopamine (9). This suggests that dobutamine is an effective inotrope with little vasopressor action. In healthy chronically instrumented newborn piglets, Cheung el al (10) found that dobutamine dose-dependently (5-50 μg/kg/min) increased heart rate and cardiac output but no effect on stroke volume. On the other hand, prolonged use (120 min) of 10 μg/kg/min of dobutamine resulted in higher cardiac output and stroke volume with transient tachycardia compared to baseline. There was no change in the systemic arterial pressure while there was a significant drop in the systemic vascular resistance. The pulmonary arterial pressure showed a dose-dependent increase but was transiently higher with the 10 μg/kg/min of dobutamine compared to baseline. There was no change in the pulmonary vascular resistance. The pulmonary to systemic arterial pressure ratio was slightly increased. However, the pulmonary arterial pressure was not suprasystemic which might be suggestive of significant pulmonary hypertension leading to reverses flow at the ductus arteriosus or patent foramen ovale.

Dobutamine and regional blood flow

There are conflicting results of the effect of dobutamine on the renal blood flow. They vary from decrease, increase or no change in blood flow (11,12). That could be explained by different conditions or methods used to study the effects. Cheung et al in the above animal study did not find any change in renal or mesenteric hemodynamics

(10). Hentschel et al used Doppler studies to examine the effect of dobutamine in the regional blood flows in hypotensive preterm infants. The found that dobutamine increased the superior mesenteric arterial flow, but it is not known whether that effect was related to the general increase in cardiac output or regional vasodilatation (13). We were not able to identify any study in the neonatal or pediatric population that examines the effect of dobutamine in cerebral blood flow. Limited literature in adults suggests no significant effect in healthy volunteers (14) or increased cerebral blood flow in septic patients receiving dobutamine (15).

Dobutamine and oxygen consumption

Inotropes increase cardiac output and systemic oxygen delivery but they might also increase the oxygen consumption (16). Penny et al studied the thermogenic effect of dobutamine in healthy newborn lams (16). They found that dobutamine increased the systemic oxygen delivery, consumption and extraction in 1-2 day old lambs more than those that are older (7-10 days and 6-8 weeks). This was also associated with an increase in body temperature by 1.3°C. This effect was not affected by giving selective β_1-, β_2-, α_1-adrenoceptor antagonists separately but minimized by giving the three together. The thermogenic effect could be due to the presence of mitochondrial uncoupling protein activation of adrenoceptors in the brown adipose tissue through nonshivering thermogenesis (17). However, it had been shown previously that the thermogenic stimulation of isolated adipocytes derived from fetal lamb by dobutamine was mainly due to the sttimulation of β_1-adrenoceptors and to a lesser extent due to α_1-adernoceptors (18). The difference could be related to the use of different models (intact animal and isolated adipocytes). In addition, some of the limitations of the study by Penny et al include the use of a healthy animal model and high dose of dobutamine infusions up to 40 μg/kg/min (16). We are not certain about the translational value of these findings because clinically, dobutamine is used in sick neonates at lower doses (5-20 μg/kg/min) (8). Moreover, human neonates have brown adipose tissue and mitochondrial uncoupling protein but to a lesser extent than that of neonatal lambs (19).

Along with increased systemic oxygen consumption, dobutamine may increase myocardial oxygen consumption (20). Therefore, the administration of dobutamine in asphyxiated neonates may be associated with significant side effects on the systemic and myocardial oxygen consumption. This metabolic effect of dobutamine in oxygen consumption has not been studied in neonatal asphyxia.

Dobutamine and platelets

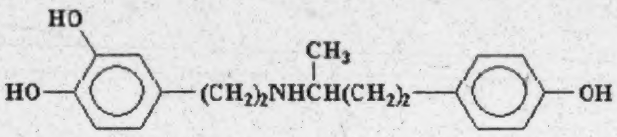
Platelet function is impaired after hypoxia and reoxygenation and may contribute to the hemostatic complications of asphyxia (21). Platelets can be activated as part of the inflammatory cascade in neonatal aphsyxia. Platelets have β-adrenoceptors on their cell membranes. Since dobutamine is a β-agonist, it can interact with these receptors and lead to activation of platelet during H-R. When inotropes are used to provide cardiovascular support during H-R, the activated and possibly exhausted platelets may also be exposed to the stimulating effects of these inotropic agents. We do not know if there is any effect of dobutamine on these receptors. In fact, there is limited literature on the interaction between dobutamine and platelet function. There is no data in the literature on the relation between dobutamine and platelet activation in the event of neonatal asphyxia.

Ganchev et al, by using different type of adrenoceptors blocking agents, found that dobutamine stimulated platelet function through the agonistic action on β_1-

adrenoceptors (22). They also concluded that the ADP-induced platelet aggregation depended to a certain extent on the functional state of the neuronal and β-adrenoreceptor unit of the adrenergic transmitter system and possibly also on the metabolic processes connected with them. In another study they also found that dobutamine increased the number of platelets (thrombocytopoiesis) (23). Combined application of practolol (a β_1-adrenergic blocking agent) and dobutamine lowered significantly the thrombocytopoiesis. In contrary to the above finding, Meyers et al (24) found that dobutamine at 0.1-10 μM inhibited ADP-induced aggregation and adding propranlol (β-antagonist) eliminated this effect.

Dobutamine stress echocardiography is used in adults to detect and evaluate patients with coronary artery disease. It is a very useful test that is cost effective and predictive of cardiac death (25). Platelet activation plays a role in cardiovascular diseases in adult such as deep venous thrombosis (26), unstable angina (27) and myocardial infarction (28). Since there are some patients who sustain nonfatal myocardial infarction related to dobutamine stress echocardiography, it was suggested that platelets could be activated by dobutamine infusion during this procedure. Galloway et al showed that there is an activation of platelets during dobutamine stress echocardiography. The activation was both dose- and time-dependent (29). The median percentage of platelet expressing CD62 (activated platelets) increased as the dose of dobutamine increased. This increase in the percentage of activated platelets was not found in patients undergoing exercise treadmill testing. However, Ditter et al (30) examined the effect of different types of stimuli on platelet rich plasma and found that dobutamine had no effect on thromboxane synthesis. Thus the mechanisms of platelet activation with the administration of dobutamine remain to be determined although there is some evidence that it may be mediated through the β-adrenoceptors.

Figure 4-1: Chemical structure of dobutamine.



Molecular weight 337.84

$C_{18}H_{23}NO_3.HCl$

4-[2-[3-(4-hydroxyphenyl)-1-methyl-propyl]aminoethyl]benzene-1,2-diol

References

1. American Society of Health-System Pharmacists 2006 Drug information. pp 1298-1301
2. Westfall TC, Westfall DP 2006 Adrenergic Agonists and Antagonists. In: Brunton LL, Parker KL, Lazo JS, Buxton ILO BD (eds) Goodman & Gilman's The Pharmacological Basis of Therapeutics.pp:237-295
3. Lehtonen LA, Antila S, Pentikainen PJ 2004 Pharmacokinetics and pharmacodynamics of intravenous inotropic agents. Clin Pharmacokinet 43:187-203
4. British Medical Association 2006 British National Formulary. BMJ Publishing Group Ltd, London, pp 117-117
5. Holmes CL 2005 Vasoactive drugs in the intensive care unit. Curr Opin Crit Care 11:413-417
6. Osborn DA 2005 Diagnosis and treatment of preterm transitional circulatory compromise. Early Hum Dev 81:413-422
7. Krivokapich J, Child JS, Walter DO, Garfinkel A 1999 Prognostic value of dobutamine stress echocardiography in predicting cardiac events in patients with known or suspected coronary artery disease. J Am Coll Cardiol 33:708-716
8. Klarr JM, Faix RG, Pryce CJ, Bhatt-Mehta V 1994 Randomized, blind trial of dopamine versus dobutamine for treatment of hypotension in preterm infants with respiratory distress syndrome. J Pediatr 125:117-122
9. Osborn D, Evans N, Kluckow M 2002 Randomized trial of dobutamine versus dopamine in preterm infants with low systemic blood flow. J Pediatr 140:183-191

10. Cheung PY, Barrington KJ, Bigam D 1999 The hemodynamic effects of dobutamine infusion in the chronically instrumented newborn piglet. Crit Care Med 27:558-564

11. Nudel DB, Peterson BJ, Buckley BJ, Gootman N, Hoffman RG 1991 Effects of acute hypoxemia on responses to dopamine and dobutamine in neonatal swine. Methods Find Exp Clin Pharmacol 13:111-119

12. Ferrara JJ, Dyess DL, Peeples GL, Christenberry DP, Roberts WS, Tacchi EJ, Swafford AN, Ardell JL, Powell RW 1995 Effects of dopamine and dobutamine on regional blood flow distribution in the neonatal piglet. Ann Surg 221:531-540

13. Hentschel R, Hensel D, Brune T, Rabe H, Jorch G 1995 Impact on blood pressure and intestinal perfusion of dobutamine or dopamine in hypotensive preterm infants. Biol Neonate 68:318-324

14. Moppett IK, Wild MJ, Sherman RW, Latter JA, Miller K, Mahajan RP 2004 Effects of ephedrine, dobutamine and dopexamine on cerebral haemodynamics: transcranial Doppler studies in healthy volunteers.Br J Anaesth 92:39-44

15. Berre J, De BD, Moraine JJ, Melot C, Kahn RJ, Vincent JL 1997 Dobutamine increases cerebral blood flow velocity and jugular bulb hemoglobin saturation in septic patients. Crit Care Med 25:392-398

16. Penny DJ, Sano T, Smolich JJ 2001 Increased systemic oxygen consumption offsets improved oxygen delivery during dobutamine infusion in newborn lambs. Inten Care Med 27:1518-1525

17. Zhao J, Cannon B, Nedergaard J 1998 Thermogenesis is beta3- but not beta1-adrenergically mediated in rat brown fat cells, even after cold acclimation. Am J Physiol 275:R2002-R2011

18. Fain JN, Mohell N, Wallace MA, Mills I 1984 Metabolic effects of beta, alpha 1, and alpha 2 adrenoceptor activation on brown adipocytes isolated from the perirenal adipose tissue of fetal lambs. Metabolism 33:289-294

19. Lean ME, James WP, Jennings G, Trayhurn P 1986 Brown adipose tissue uncoupling protein content in human infants, children and adults. Clin Sci (Lond) 71:291-297

20. Chandler MP, Stanley WC, Morita H, Suzuki G, Roth BA, Blackburn B, Wolff A, Sabbah HN 2002 Short-term treatment with ranolazine improves mechanical efficiency in dogs with chronic heart failure. Circ Res 91:278-280

21. Cheung PY, Stevens JP, Haase E, Stang L, Bigam DL, Etches W, Radomski MW 2006 Platelet dysfunction in asphyxiated newborn piglets resuscitated with 21% and 100% oxygen. Pediatr Res 59:636-640

22. Ganchev T, Negrev N, Ilkova R 1990 Adrenergic dependence of the platelet aggregation in rats. Acta Physiol Pharmacol Bulg 16:46-49

23. Ganchev TS, Negrev NN 1989 Beta1- and beta2-adrenergic effects on thrombocytopoiesis in rats. Gematol Transfuziol 34:50-53

24. Meyers KM, Huston LY, Clemmons RM 1983 Regulation of canine platelet function II. Catecholamines. Am J Physiol 245:R100-R109

25. Sicari R, Pasanisi E, Venneri L, Landi P, Cortigiani L, Picano E 2003 Stress echo results predict mortality: a large-scale multicenter prospective international study. J Am Coll Cardiol 41:589-595

26. Metzelaar MJ, Korteweg J, Sixma JJ, Nieuwenhuis HK 1991 A Comparative-Study on the Use of Platelet Membrane Markers for the Detection of Platelet Activation Invitro and in Clinical Disorders. Throm and Haemost 65:680

27. Pastan I, Willingham MC, FitzGerald DJ 1986 Immunotoxins. Cell 47:641-648

28. Vesterqvist O 1988 Measurements of the in vivo synthesis of thromboxane and prostacyclin in humans. Scand J Clin Lab Invest 48:401-407

29. Galloway MT, Paglieroni TG, Wun T, Arena FJ, Lewis WR 1998 Platelet activation during dobutamine stress echocardiography. Am Heart J 135:888-900

30. Ditter H, Matthias FR, Lasch HG. 1984 Comparison of sympathomimetic amines with collagen, calcium ionophor and adenosine diphosphate in their effects on thromboxane formation of human platelet rich plasma. Folia Haematol Int Mag Klin Morphol Blutforsch.111:761-8.

CHAPTER 5

Animal models of hypoxia and reoxygenation

Introduction

Studying neonatal asphyxia can be quite challenging for many factors. These factors may include: difficulty in predicting the onset and severity of asphyxia. It is also difficult and even unethical to get some biological specimens from the asphyxiated neonates. The temporal changes can also be difficult to monitor. These factors make the animal studies alternative to the asphyxiated human neonates. Therefore, much of our understanding of neonatal asphyxia has been extrapolated from animal studies. Fetal and neonatal sheep, pigs, rodents and others have been used to study perinatal asphyxia. No model is considered to be perfect because of the species differences between human neonates and animal young pups. In addition, the type of injury or insult used in animal models might not be the same as those in human neonates. Subsequently, the responses to the injury might be different as well. Nevertheless, animal studies have provided us with great amount of knowledge regarding neonatal asphyxia.

Types of neonatal asphyxia model

There are various animal models with different types of insults and degrees of severity that have been developed to study neonatal asphyxia. Raju summarized these models (1). Table 5-1 shows the common methods used to induce neonatal asphyxia in animal studies. They include antenatal methods in which restricting of blood flow to the fetus is the main type of insults. This can be achieved by occluding the blood supply to the uterus by compressing maternal uterine artery (2) or abdominal aorta (3), or inducing maternal hypotension by bleeding (4) or administration of drugs 4). Blood supply to the fetus can also be compromised by placental abruption (5) and umbilical cord occlusion (6). It is important to mention that some studies included fetal instrumentation to study the effect of perinatal asphyxia in a few days after surgery (7). These methods provide an opportunity to study perinatal asphyxia after a reasonable period of stabilization and healing.

Postnatally, asphyxia can be induced mainly by alveolar hypoxia (8). Alveolar hypoxia can be achieved by reducing ambient oxygen concentration/partial pressure below atmospheric pressure and this is applied mainly in small animals (9). In bigger animals like piglets and sheep, alveolar hypoxia is usually achieved by ventilating animals with decreased inspired oxygen concentration (10). In addition, decreasing the ventilatory rate or even withdrawal of ventilatory support temporarily can be used to achieve some degree of respiratory failure and even cardio-respiratory arrest (11,12). Following phlebotomy, neonatal hypovolemia with shock and hypotension has also been used (13). Occlusion of the common carotid artery is used mainly to study the local effect of cerebral ischemia (14). However, this model lacks the systemic changes that usually accompany neonatal asphyxia. Thus, usually the occlusion of the common carotid artery is combined with alveolar hypoxia in neonatal rat pups to study hypoxic ischemic encephalopathy.

In vitro studies are also utilized to study the effect of asphyxia on isolated organs and tissue culture. The effect of ischemia-reperfusion and hypoxia-reoxygenation on isolated hearts has been studied extensively (15). *In vitro* and *ex vivo* studies of platelet function have been used to investigate the effects of ischemia-reperfusion and hypoxia-reoxygenation on platelets. Isolated cells of different body organs have also been studied in response to hypoxia and reoxygenation (16).

Similarities between piglets and human

The body weight and size of piglets match with those of near-term (36-38 week gestation) neonates, and this makes newborn piglets a very attractive model. At birth, newborn pigs weigh approximately 1.5-2 kg which makes various intervention and instrumentation feasible. They are tolerant to various interventions such as surgery, anesthetics and hypoxia when compared to other species (17). They also mature at comparable rate to that of human neonates (17). The easy availability and relatively low cost also makes it feasible to perform research on this species. More importantly, the similarities in physiology and anatomy between neonatal pigs and human neonates allow relevant interpretation of the findings (17). There is a considerable amount of literature on the use of newborn pigs in perinatal research (18).

Newborn piglets have contributed extensively to the cardiovascular research due to the similarities in hemodynamic parameters such as heart rate, blood pressure, cardiac output, stroke volume and regional blood flow with human neonates (19,20). The hemodynamic changes to perinatal asphyxia are also similar to those of human neonates (21). Although several studies have shown the absence of significant blood flow across the ductus arteriosus, the ductus arteriosus may need to be carefully identified and ligated. This is done to prevent any possible shunting of the blood flow that may occur during the experimental period as a response to increased pulmonary vascular resistance during hypoxia. It is however important to mention that shunting through the patent foramen ovale can not be excluded. An echocardiography may be needed to assess this type of intra-cardiac shunting.

The pulmonary vasculature of newborn piglets has been studied and shown to have a similar fashion of postnatal development although the maturation in piglets is rapid (22). Nonetheless, similar pulmonary vasoconstriction is observed in the response to hypoxia (21) .

Piglet experiments have contributed greatly to the understanding of acute effects of various components of perinatal asphyxia on cerebral blood flow and metabolism. Many studies provide insight into the complex histological, biochemical and vascular responses of perinatal asphyxia and a variety of pharmacologic stimuli and other interventions. Hypothermia to treat hypoxic ischemic encephalopathy has been used in newborn piglets (23). Long-term survival model were also studied (24).

The development and maturation of intestinal tract is also similar to that in human neonates (25,26). This makes it a good model to study the mesenteric circulation during perinatal asphyxia. Necrotizing enterocolitis, a common complication of perinatal asphyxia, can also develop in piglets as a result of hypoxia and reoxygenation (10). The piglet liver also shares similar ultrastructure with human (27).

Pigs are one of few species that have similar renal structure as human (28). They share similar renal physiology and fetal-neonatal development (28). They also have similar serum chemistry and acid-base balance as in human (29).

Hypoxia and reoxygenation models in newborn piglets

Hypoxia and reoxygenation has been used extensively in perinatal research to study the effect of asphyxia on neonates (10,30). Severe hypoxia may lead to ischemia due to severe shock and hypotension and blood flow redistribution (10).

In our model, after stabilization, we induced hypoxia for 2h. We believe the duration of hypoxia mimics the clinical scenario. In clinical practice, from the time of signs of fetal distress (e.g. tachycardia) are identified during the intrapartum period, to diagnosis and subsequent arrangement of emergency cesarean section be performed, this will eventually take 1-2h to deliver the baby and initiate resuscitation. It is true if the fetal distress is very ominous such as bradycardia as at the end of 2h of hypoxia, the preparation of all these procedures will probably take place much faster. We adjusted the severity of hypoxia (10-15% oxygen) to cause severe metabolic acidosis and a significant decrease of systemic arterial pressure and cardiac output by 50% from baseline.

The reoxygenation mimics the resuscitation in asphyxiated neonates. Drugs (31) and volume (32) can be given during this stage to study the effect of such interventions after resuscitation. It is essential to avoid or at least to control drugs used to help in salvaging the asphyxiated animals when other interventions are being examined.

Various animal models were used to study the differences between room air and 100% oxygen for resuscitation (33,34). There is still debate regarding the appropriate oxygen concentration to be used in neonatal resuscitation. The aim of this study was not to study these differences. Therefore we followed recent guidelines by the American Heart Association for the resuscitation of term neonates and used 100% oxygen (35). This was given for 1h following the hypoxia. The duration of using 100% oxygen might be prolonged. However, in clinical practice most asphyxiated neonates are born in community hospitals. After resuscitation, these neonates may be kept at 100% oxygen until the transport team arrives and adjusts the oxygen concentration accordingly. For the remaining period of reoxygenation, we kept the inspired oxygen concentration between 21% and 25% to maintain systemic arterial saturation of 90-95%.

Anesthesia was induced using halothane which may have cardiovascular side effects. These side effects may include bradycardia and hypotension (36). In order to avoid potential cardiovascular depression, halothane was given for only a short period of time (<20 min) and switched to i.v. drug infusions once venous access and airway were established. Fentanyl (an analgesic drug), midazolam (a sedative drug), pancuronium (muscle relaxant) and acepromazine (a major tranquilizer) were given to control pain and body movements. These drugs are used in the management of the sick neonates at similar doses. They have been proven to be safe with minimum side effects and well tolerated by the newborn piglets (37).

Dobutamine was given at 2h of reoxygenation which is around the time of at which myocardial stunning develops. In clinical practice, dobutamine is given to treat hypotension following neonatal asphyxia. Therefore, we aimed to give dobutamine around the same time that myocardial stunning and hypotension may develop.

Conclusions

In summary, we used a well established neonatal swine model of hypoxia and reoxygenation that mimics asphyxia and resuscitation in the human neonates. We examine the effect of dobutamine on hypotension and myocardial dysfunction that usually develop following the resuscitation of neonates.

Choosing the appropriate model, recognizing the limitations of the model and sound interpretation of data are very important issues that need to be addressed before applying the results generated from animal studies to humans.

Table 5-1: Commonly used methods of inducing perinatal/neonatal asphyxia (1).

- Antenatal (intrauterine, fetal)
  - Maternal hypoxia
  - Maternal hypotension by halothane; bleeding
  - Oxytocin to increase uterine contractions
  - Constricting maternal abdominal aorta
  - Uterine artery occlusion
  - Placental embolization
  - Umbilical cord compression
- Postnatal (neonatal)
  - Alveolar hypoxia with or without hypercapnia
  - Carotid artery occlusion with or without alveolar hypoxia
  - Bleeding
  - Respiratory arrest

References

1. Raju TN 1992 Some animal models for the study of perinatal asphyxia. Biol Neonate 62:202-214
2. Gunn AJ, Parer JT, Mallard EC, Williams CE, Gluckman PD 1992 Cerebral histologic and electrocorticographic changes after asphyxia in fetal sheep. Pediatr Res 31:486-491
3. Jensen A, Hanson MA 1995 Circulatory responses to acute asphyxia in intact and chemodenervated fetal sheep near term. Reprod Fertil Dev 7:1351-1359
4. Calvert SA, Widness JA, Oh W, Stonestreet BS 1990 The effects of acute uterine ischemia on fetal circulation. Pediatr Res 27:552-556
5. Myers RE, Brann AW, Jr. 1976 Abruptio placentae in rhesus monkey causing brain damage to the fetus. Am J Obstet Gynecol 126:1048-1049
6. Myers RE 1975 Fetal asphyxia due to umbilical cord compression. Metabolic and brain pathologic consequences. Biol Neonate 26:21-43
7. Gunn AJ, Maxwell L, De Haan HH, Bennet L, Williams CE, Gluckman PD, Gunn TR 2000 Delayed hypotension and subendocardial injury after repeated umbilical cord occlusion in near-term fetal lambs. Am J Obstet Gynecol 183:1564-1572
8. Nioka S, Chance B, Smith DS, Mayevsky A, Reilly MP, Alter C, Asakura T 1990 Cerebral energy metabolism and oxygen state during hypoxia in neonate and adult dogs. Pediatr Res 28:54-62
9. Jensen FE, Applegate CD, Holtzman D, Belin TR, Burchfiel JL 1991 Epileptogenic effect of hypoxia in the immature rodent brain. Ann Neurol 29:629-637

10. Haase E, Bigam DL, Nakonechny QB, Jewell LD, Korbutt G, Cheung PY 2004 Resuscitation with 100% oxygen causes intestinal glutathione oxidation and reoxygenation injury in asphyxiated newborn piglets. Ann Surg 240:364-373
11. Ment LR, Stewart WB, Duncan CC, Pitt BR 1987 Beagle pup model of perinatal asphyxia: nimodipine studies. Stroke 18:599-605
12. Hansen NB, Brubakk AM, Bratlid D, Oh W, Stonestreet BS 1984 The effects of variations in PaCO2 on brain blood flow and cardiac output in the newborn piglet. Pediatr Res 18:1132-1136
13. Matsuda H, Raju TN, Maeta H, John E, Fornel L, Vidyasagar D 1988 Effect of acute hypovolemic hypotension on cerebral metabolism in newborn piglets. Brain Dev 10:13-19
14. Aden U, Dahlberg V, Fredholm BB, Lai LJ, Chen Z, Bjelke B 2002 MRI evaluation and functional assessment of brain injury after hypoxic ischemia in neonatal mice. Stroke 33:1405-1410
15. Fisher DJ 1984 Increased regional myocardial blood flows and oxygen deliveries during hypoxemia in lambs. Pediatr Res 18:602-606
16. Zovein A, Flowers-Ziegler J, Thamotharan S, Shin D, Sankar R, Nguyen K, Gambhir S, Devaskar SU 2004 Postnatal hypoxic-ischemic brain injury alters mechanisms mediating neuronal glucose transport. Am J Physiol Regul Integr Comp Physiol 286:R273-R282
17. Swindle MM 1985 Porcine models in surgical research: An overview in Swine in Biomedical Research. Plenum Press, New York, pp 235-242

18. Hill DE 1985 Swine in perinatal research: an overview In: Tumbleson ME (ed) Swine in Biomedical Research. Plenum Press, New York, pp 1155-1159

19. Lee KT 1985 Swine as animal model in cardiovascular research In: Tumbleson ME (ed) Swine in biomedical Research. Plenum Press, New York, pp 1481-1496 .

20. Hannon JP 1985 Swine as animal model in cardiovascular research In: Tumbleson ME (ed) Swine in Biomedical Research. Plenum Press, New York, pp -1341

21. Alonso-Spilsbury M, Mota-Rojas D, Villanueva-Garcia D, Martinez-Burnes J, Orozco H, Ramirez-Necoechea R, Mayagoitia AL, Trujillo ME 2005 Perinatal asphyxia pathophysiology in pig and human: a review. Anim Reprod Sci 90:1-30

22. Redding GJ, Truog WE 1986 Pulmonary vascular reactivity and gas exchange in response to global and regional hypoxia in newborn piglets In: Tumbleson ME (ed) Swine in Biomedical Research. Plenum press, New york, pp 1187-1194

23. Thoresen M, Simmonds M, Satas S, Tooley J, Silver IA 2001 Effective selective head cooling during posthypoxic hypothermia in newborn piglets. Pediatr Res 49:594-599

24. Thoresen M, Haaland K, Loberg EM, Whitelaw A, Apricena F, Hanko E, Steen PA 1996 A piglet survival model of posthypoxic encephalopathy. Pediatr Res 40:738-748

25. Touloukian RJ, Posch JN, Spencer R 1972 The pathogenesis of ischemic gastroenterocolitis of the neonate: selective gut mucosal ischemia in asphyxiated neonatal piglets. J Pediatr Surg 7:194-205

26. Widdowson EM 1976 Changes in the body and its organs during lactation: nutritional implications. Ciba Found Symp103-118

27. Singh A 1986 Histotology of piglet liver. In: Tumbleson ME (ed) Swine in Biomedical Research Plenum Press, New York, pp 77-87

28. Terris JM 1986 Swine model in renal physiology and nephrology In: Tumbleson ME (ed) Swine in Biomedical Research. Plenum Press, New York, pp 1673-1689

29. Tumbleson ME, Schmidt DA 1986 Swine clinical chemistry In: Tumbleson ME (ed) Swine in Biomedical Research. Plenum Press, New York, pp 783-807

30. Medbo S, Yu XQ, Asberg A, Saugstad OD 1998 Pulmonary hemodynamics and plasma endothelin-1 during hypoxemia and reoxygenation with room air or 100% oxygen in a piglet model. Pediatr Res 44:843-849

31. Ferrara JJ, Dyess DL, Peeples GL, Christenberry DP, Roberts WS, Tacchi EJ, Swafford AN, Ardell JL, Powell RW 1995 Effects of dopamine and dobutamine on regional blood flow distribution in the neonatal piglet. Ann Surg 221:531-540

32. Liu X, Nozari A, Rubertsson S, Wiklund L 2002 Buffer administration during CPR promotes cerebral reperfusion after return of spontaneous circulation and mitigates post-resuscitation cerebral acidosis. Resuscitation 55:45-55

33. Borke WB, Munkeby BH, Halvorsen B, Bjornland K, Tunheim SH, Borge GI, Thaulow E, Saugstad OD 2004 Increased myocardial matrix metalloproteinases in hypoxic newborn pigs during resuscitation: effects of oxygen and carbon dioxide. Eur J Clin Invest 34:459-466

34. Haase E, Bigam DL, Nakonechny QB, Rayner D, Korbutt G, Cheung PY 2005 Cardiac function, myocardial glutathione, and matrix metalloproteinase-2 levels in hypoxic newborn pigs reoxygenated by 21%, 50%, or 100% oxygen. Shock 23:383-389

35. American Heart Association 2005 Part 13: Neonatal Resuscitation Guidelines. Circulation 112:IV-188

36. Ciofolo MJ, Reiz S 1999 Circulatory effects of volatile anesthetic agents. Minerva Anestesiol 65:232-238

37. Kaiser GM, Heuer MM, Fruhauf NR, Kuhne CA, Broelsch CE 2006 General handling and anesthesia for experimental surgery in pigs. J of Surg Res 130:73-79

CHAPTER 6

The hemodynamic effects of dobutamine during reoxygenation after hypoxia: a dose-response study in newborn pigs

Abstract

Introduction: Dobutamine is commonly used to treat shock and hypotension developed after the resuscitation of asphyxiated neonates. Using a neonatal swine model of hypoxia-reoxygenation, we examined the dose-response effect of dobutamine (5-20 μg/kg/min) on systemic and regional circulations and oxygen metabolism.

Methods: Newborn piglets were acutely instrumented for continuous monitoring of heart rate, systemic and pulmonary arterial pressures, and pulmonary (surrogate for cardiac index), right common carotid, superior mesenteric and left renal arterial flows. After stabilization they were exposed to normocapnic alveolar hypoxia (10-15% oxygen) for 2h followed by reoxygenation with 100% oxygen for 1h then 21% for 3h. Piglets were block-randomized to receive dobutamine infusion (5, 10 or 20 μg/kg/min) or saline (control) at 2-4h of reoxygenation (n=8 each).

Results: Two hours of hypoxia followed by reoxygenation resulted in hypotension and myocardial dysfunction. Cardiac index improved significantly with 20 μg/kg/min of dobutamine and modestly in the 5 and 10 μg/kg/min treatment groups (at 120 min: 172±35, 160±30 and 158±56 vs. 119±33 ml/kg/min of controls, $p<0.05$, respectively), with corresponding increases in stroke volume. Pulmonary vascular resistance was lower in all dobutamine-treated groups (vs. controls $p<0.05$). There were no differences in heart rate, systemic and pulmonary arterial pressures, systemic vascular resistance and regional flows between groups. The group of 20 μg/kg/min of dobutamine had also higher systemic oxygen delivery (at 2h of dobutamine: 18±5 vs. 11±3 ml O_2/kg/min of controls, $p<0.05$) with no significant differences in systemic oxygen consumption and regional oxygen delivery between groups.

Conclusions: Following the reoxygenation of newborn piglets with severe hypoxia, high dose of dobutamine is effective to treat myocardial stunning and low cardiac output with no significant effect on blood pressure or regional circulation.

Introduction

Neonatal asphyxia is often complicated by multi-organ dysfunction (1) that can lead to death (2,3) and serious long-term sequelae including cerebral palsy (4). Soon after reoxygenation from severe asphyxia, myocardial dysfunction develops (5) which may be related to the myocardial stunning due to oxidative stress-related injury following ischemia-reperfusion (6) or hypoxia-reoxygenation (H-R) (7,8). In addition, there are other cardiovascular manifestations such as pulmonary hypertension and poor regional perfusion (9). Post-asphyxial myocardial dysfunction is often treated by inotropes to enhance cardiac output (10). Dobutamine, dopamine and epinephrine are three commonly used inotropes. Unfortunately, there is no consensus regarding the best inotropes in asphyxiated neonates (11). In the meta-analysis of therapies in hypotensive preterm neonates, Cochrane Systematic Reviews conclude that dopamine is more effective than volume expansion (12) and dobutamine (13) in improving systemic arterial pressure (SAP) with no significant effects on cardiac output. However, the use of dopamine may also aggravate pulmonary hypertension (14), intestinal ischemia (15) and decrease cardiac output (16). Epinephrine is efficient in treating systemic hypotension (17). However, data are insufficient to support the routine use of epinephrine to prevent morbidity or mortality in premature neonates (18). Beside the lack of data in term asphyxiated neonates, there is concern regarding vasoconstriction. Epinephrine can aggravate systemic and pulmonary vasoconstriction and increase lactate production (19-21).

Dobutamine, a semi-synthetic sympathomimetic, has β-adrenoceptor effects that are accompanied by some α-adrenoceptor effects (16,22). It has been suggested that dobutamine is better than dopamine in increasing the cardiac output (16) but less effective in correcting the SAP (13). Although Crowley et al showed an 11% decrease in pulmonary arterial pressure (PAP) with dobutamine at 5-10 µg/kg/min (23), a dose of 20 µg/kg/min or greater can cause significant pulmonary hypertension and possible mesenteric (24) and renal vasoconstriction (25) in normoxic neonatal piglets. Because of the compromised systemic and regional circulations in neonates after H-R, it is critical to choose an appropriate inotrope to treat the hypotension and shock during reoxygenation. Little information is available on the effects of dobutamine infusion regarding hypotension and myocardial stunning, regional blood flow and oxygen transport following the reoxygenation of asphyxiated neonates.

Therefore, we designed this experiment to study the effect of dobutamine on systemic and regional circulations during reoxygenation after hypoxia in newborns. We used an established neonatal swine model of H-R (26) that mimics the clinical scenario of H-R with 100% oxygen. We also studied the effect of dobutamine on oxygen transport and plasma lactate concentration. We hypothesized that dobutamine would dose-dependently increase the cardiac output and SAP with no adverse effects on regional circulations in neonatal H-R.

Methods

The experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Health Science Animal Policy and Welfare Committee, University of Alberta.

Animal preparation

Thirty-eight mixed-breed newborn piglets (age: 1-3 day, weight: 1.5-2.3 kg) were brought in from a local farm on the same day of experimentations. Piglets were anesthetized initially with halothane while spontaneously breathing and then given i.v. fentanyl (5-15 μg/kg/h), midazolam (0.1-0.2 mg/kg/h), and pancuronium (0.05-0.1 mg/kg/h) once the i.v. access was secured and the animal was mechanically ventilated through tracheostomy. Additional doses of fentanyl (10 μg/kg) and pancuronium (0.1 mg/kg) were given as needed. A dose of i.v. acepromazine (0.25 mg/kg) was also given. The above drugs are usually given to the critically sick neonates.

The animals were ventilated by a Sechrist infant ventilator (Model IV-100; Sechrist Industries, Anaheim, CA) set at pressures of 18/4 cm H_2O, rates of 15-20 breaths/min, and fractional inspired oxygen concentration (FiO_2) of 0.21-0.25. The aim was to maintain oxygen saturation of 90% to 100% and $PaCO_2$ of 35-45 mm Hg. Oxygen saturation was continuously monitored with a pulse oximeter (Nellcor, Hayward, CA).

Right femoral arterial (single lumen) and venous (double lumen) catheters (3.5 or 5 Fr; Sherwood Medical Co., St. Louis, MO) were inserted to the level of infra-renal aorta and right atrium, respectively. The arterial catheter was connected to a pressure transducer to monitor mean SAP and used for blood sampling. The venous catheter was used to monitor central venous pressure (CVP) and the administration of medications and fluids. Heart rate and SAP were measured with a Hewlett Packard 78833B monitor (Hewlett Packard Co., Palo Alto, CA). Maintenance fluid consisted of 10% dextrose-saline at 10 ml/kg/h.

Through a midline neck incision and after establishing an airway by tracheostomy, the right common carotid artery (CA) was isolated and a 2-mm transit time ultrasound flow probe (2SS, Transonic Systems Inc., Ithica, NY) was placed around it. A left anterior thoracotomy was performed in the 4th intercostal space. The ductus arteriosus was ligated followed by inserting a 20-gauge catheter into the root of the pulmonary artery for the measurement of mean pulmonary artery pressure (PAP). A 6-mm Transonic flow probe (6SB) was placed around the main pulmonary artery to measure the cardiac output continuously. The retroperitoneum was opened via a left flank incision. The superior mesenteric (SMA) and left renal (RA) arteries were isolated and encircled with a 3-mm (3SB) and a 2-mm (2SB) Transonic flow probes, respectively. All incisions were covered to minimize evaporative heat loss. Heart rate, cardiac output, SAP, PAP, CVP and CA, SMA and RA flows were continuously monitored. Analogue outputs of the pressure amplifiers and flow monitors were digitized by a DT 2801-A analogue to digital converter board (Data Translation, Ontario, Canada) in a Dell 425E personal computer. Software was custom written using the Asyst programming environment.

The piglet's temperature was maintained at 38.5-39.5°C using an overhead warmer and a heating pad. Stabilization, a minimum of 30 min after surgery finished, was defined as $\leq$10% change in heart rate and SAP, normal arterial blood gases and acid-base balance (PaO_2 60-100 mmHg; $PaCO_2$ 35-55 mmHg; pH 7.35-7.45).

Experimental protocol

Piglets were block-randomized into four groups. After stabilization, normocapnic alveolar hypoxia (FiO_2=0.10-0.15) was induced in piglets for 2h, by increasing the concentration of inhaled nitrogen gas, aiming for arterial oxygen saturations of 30-40%

and severe metabolic acidosis. This was followed by reoxygenation with 100% oxygen for 1h and then 21% oxygen for 3h. At 2h of reoxygenation, either dobutamine (Dobutrex, Eli Lilly Inc., Scarborough, ON, Canada) at a fixed dose (5, 10, or 20 μg/kg/min) or saline (control) was given (n=8 in each of these H-R group) for a total duration of 2h. Sham piglets (n=6) received saline and were not exposed to H-R. At the end of the study, piglets were euthanized with an i.v. injection of pentobarbital (100 mg/kg).

Hemodynamic measurements and oxygen transport

Hemodynamic recordings for data analysis were carried out at specified timepoints: baseline, 120 min (2h of hypoxia), 130 min (10 min reoxygenation), 240 min (2h reoxygenation, pre-dobutamine), 270, 300, 330 and 360 min (30, 60, 90 and 120 min of dobutamine infusion, respectively). The cardiac output, CA, SMA and RA blood flows were indexed for piglet weight and expressed as ml/kg/min. The following variables were calculated:

1. Stroke volume index (SVI) (ml/kg/beat) = Cardiac index (CI) ÷ Heart rate.
2. Systemic vascular resistance index (SVRI) (mmHg/ml/min/kg) = (SAP - CVP) ÷ CI.
3. Pulmonary vascular resistance index (PVRI) (mmHg/ml/min/kg) was estimated by PAP ÷ CI.

The ratio of PAP/SAP was also calculated and analyzed because this ratio may reflect the relative pressure gradient and thus the direction of shunting across the ductus arteriosus in the transitional pulmonary circulation of sick neonates.

Simultaneous blood samples were drawn for determination of blood gases, arterial and mixed venous oxygen saturation by ABL500 and OSM 3 Hemoximeter (Radiometer, Copenhagen, Denmark) at the specified timepoints as above, respectively. Acid-base balance and hemoglobin concentration were also determined and the following were calculated:

1. Systemic oxygen delivery ($sysDO_2$) (ml O_2/kg/min) = CI x SaO_2 x 1.34 x [Hb].
2. Systemic oxygen consumption ($sysVO_2$) (ml O_2/kg/min) = CI x (SaO_2 - SvO_2) x 1.34 x [Hb].
3. Systemic oxygen extraction ($sysEO_2$) (%) = [(SaO_2 - SvO_2) ÷ SaO_2] x 100%.
4. Carotid arterial oxygen delivery ($CADO_2$) (ml O_2/kg/min) = CA flow index (FI) x SaO_2 x 1.34 x [Hb].
5. Superior mesenteric arterial oxygen delivery ($SMADO_2$) (ml O_2/kg/min) =SMA FI x SaO_2 x 1.34 x [Hb].
6. Renal arterial oxygen delivery ($RADO_2$) (ml O_2/kg/min) = RA FI x SaO_2 x 1.34 x [Hb].

Determination of plasma lactate

Platelet poor plasma was prepared by centrifugation at 10,000g for 15 min and stored at -80°C. The plasma lactate concentration was measured by enzyme linked metabolite assay of NADH with spectrophotometry at 340 nm (27).

Statistical analysis

Results are expressed as mean±SD. Two-way analysis of variance was used to identify the difference between groups at different doses. One-way analysis of variance was also used to compare differences of means between groups at each time point with

Fisher's least significant difference test for *post hoc* analysis. Kruskal-Wallis test with Dunn's test for *post hoc* analysis was used to compare the differences between groups for nonparametric variables. A statistic software (SigmaStat, v.2.0, Jandel Corp., San Rafael, CA) was used. Significance was defined as $p<0.05$. A sample size of 8 animals in was required each group based on type 1 error of 0.05, power of 0.8% and an increase in CI by 50% in the intervention groups.

Results

The animals were 2.0±0.9 day old, weighing 1.80±0.20 kg with no significant differences between groups. All groups were similar at baseline in all hemodynamic variables and hemoglobin concentration (mean 7.7-8.9 g/dl). The piglets had normal blood gases and acid-base balance (Table 6-1). Sham-operated animals were stable throughout the experimental period.

Two hours of hypoxia (mean PaO_2: 31-36 mmHg and mean SaO_2: 33-40%) resulted in a severe metabolic acidosis (mean pH: 7.02-7.06) and increased plasma lactate concentration (mean 14.1-15.0 mmol/L) (Tables 6-1 and 6-2). The arterial pH returned back to normal after 2h of reoxygenation (mean PaO_2: 62-69 mmHg and mean SaO_2: 90-91%) (Table 6-1). There were no differences between the dobutamine-treated and control groups in the acid-base balance and plasma lactate concentrations at the specified timepoints during the experimental period (Tables 6-1 and 6-2).

Systemic hemodynamic responses

Severe normocapnic alveolar hypoxia resulted in cardiogenic shock (mean CI: 84-105 ml/kg/min and mean SVI: 0.43-0.56 ml/kg/beat vs. 165±41 ml/kg/min and 0.89±0.29 ml/kg/beat of shams, $p<0.05$, respectively) (Figures 6-1 and 6-2). The CI recovered within 10 min of reoxygenation (mean CI: 157-185 ml/kg/min) then declined gradually during further reoxygenation (mean CI at 2h of reoxygenation: 134-156 ml/kg/min). Dobutamine infusion dose-dependently increased CI with significantly higher CI of the 20 μg/kg/min dobutamine-treated group than those of controls (at 30 min of infusion: 188±37 vs. 131±31 ml/kg/min of controls; at 120 min of infusion: 172±35 vs. 119±33 ml/kg/min of controls, both $p<0.05$) (Figure 6-1). The improvement in the 5 and 10 μg/kg/min dobutamine-treated groups was intermediate.

All H-R animals had similar reduction in SVI after hypoxia and similar recovery at 10 min of reoxygenation (mean SVI: 0.72-0.83 ml/kg/beat). At 2h of reoxygenation, the mean SVI of H-R groups were 0.60-0.74 ml/kg/beat (Figure 6-2). The increase in CI of 20 μg/kg/min dobutamine-treated group was associated with a better SVI that was significantly higher than those of controls, whereas the SVI of 5 and 10 μg/kg/min dobutamine-treated groups were intermediate.

There were no significant differences in heart rate between all groups at baseline or at the end of hypoxia (Figure 6-3). At 2h of reoxygenation and prior to dobutamine infusion, the group of 10 μg/kg/min of dobutamine had higher heart rate than control ($p<0.05$). Dobutamine infusion did not cause any changes in the heart rate compared to controls ($p>0.05$) although all H-R animals remained tachycardia during reoxygenation (vs. 183-194 beats/min of shams at 2-4h of reoxygenation, $p<0.05$).

At the end of 2h hypoxia, there was severe hypotension (mean SAP: 27-36 mmHg). The SAP recovered after 10 min of reoxygenation (mean SAP: 49-58 mmHg) (Figure 6-4). Subsequently, the mean SAP of H-R groups gradually declined to 40-48 mmHg at 2h of reoxygenation. The infusion of dobutamine did not affect SAP, which was not different between groups (Figure 6-4).

All H-R animals had severe hypoxia-induced pulmonary hypertension (mean PAP: 36-40 vs. 27±4 mmHg in shams at 2h of hypoxia, $p<0.05$) with increased PAP/SAP ratio that persisted in spite of 10 min of reoxygenation (Figures 6-5 and 6-6). The increased PAP normalized after 2h of reoxygenation but the PAP/SAP ratio remained higher than sham (mean PAP/SAP ratio: 0.66-0.82 vs. 0.52±0.04 of shams, $p<0.05$) (Figures 6-5 and 6-6). There was a modest increase in the PAP of the H-R control group with no significant difference between groups at 4h of reoxygenation (120 min of dobutamine or saline infusion). However, the PAP/SAP ratio was maintained below one during the 120 min infusion in all dobutamine-treated groups (mean PAP/SAP ratio: 0.79-0.85 vs. 1.00 of controls, $p=0.08$, $\beta=0.65$) (Figure 6-6).

By the end of hypoxia, the SVRI decreased modestly (Figure 6-7) while the PVRI increased significantly (Figure 6-8) in all H-R groups. There were no significant differences between groups during the first 2h of reoxygenation. The SVRI was slightly lower in the dobutamine-treated groups than those of controls but that did not reach statistical significance ($p=0.1$, $\beta=0.73$). On the other hand, all dobutamine-treated groups had lower PVRI than that of controls ($p<0.05$).

Systemic oxygen transport

The $sysDO_2$, $sysVO_2$ and $sysEO_2$ were similar at baseline in all groups. The $sysDO_2$ decreased significantly with alveolar hypoxia (Figure 6-9) and associated with decreased $sysVO_2$ (Figure 6-10) and increased $sysEO_2$ (Figure 6-11). The $sysDO_2$ recovered immediately within 10 min of reoxygenation but decreased when FiO_2 changed from 1.00 to 0.21 during reoxygenation. On the other hand, the sysVO$_2$ remained low at 10 min of reoxygenation (vs. 6.8±0.4 ml O$_2$/kg/min of shams, $p<0.05$) and gradually improved to levels that were not different from those of shams at 2h of reoxygenation (Figure 6-10). There was also a significant increase in the sysEO$_2$ as a result of 2h of hypoxia (vs. 38±7% of shams, $p<0.05$) which decreased immediately upon reoxygenation ($p<0.05$ vs. sham) but again increased prior to the start of dobutamine (vs. 37±6% of shams $p<0.05$) (Figure 6-11). At 30 min of dobutamine infusion, the 20 μg/kg/min dobutamine-treated group had higher sysDO$_2$ than that of controls (20±6 vs. 12±2 ml O$_2$/kg/min of controls, $p<0.05$) (Figure 6-9). This significant difference persisted during 120 min of dobutamine infusion. The effect of 5 and 10 μg/kg/min dobutamine on sysDO$_2$ was modest. Furthermore, there were no significant differences in the sysVO$_2$ ($p=0.21$) and sysEO$_2$ ($p=0.65$) between groups (Figures 6-10 and 6-11).

The plasma lactate concentrations were similar at baseline (Table 6-2). At 2h of hypoxia, the plasma lactate concentrations were higher in the H-R groups than those of sham-operated animals and remained significantly higher in spite of 2h of reoxygenation (vs. 3.6±1.1 mmol/L of shams, $p<0.05$). All H-R groups had similar plasma lactate concentrations at 4h of reoxygenation (120 min of dobutamine or saline infusion) which was not different from sham (vs. 3.5±1.5 mmol/L of shams, $p>0.05$)

Regional hemodynamic responses and oxygen delivery

After 2h of hypoxia, the CA FI was preserved with no significant differences between groups (Figure 6-12). No significant changes in CA FI were observed during reoxygenation. The CADO$_2$ decreased significantly and similarly in all H-R groups during hypoxia (vs. shams, $p<0.05$), which recovered immediately with reoxygenation

(Figure 6-13). The infusion of dobutamine did not result in any significant change on CA FI or $CADO_2$.

Two hours of hypoxia resulted in decreased SMA FI and $SMADO_2$ ($p=0.05$ and $p<0.001$ vs. shams, respectively) (Figures 6-14 and 6-15). Upon reoxygenation, both SMA FI and $SMADO_2$ increased. The infusion of 20 μg/kg/min had transient and modest increases in SMA FI (at 30-60 min, $p=0.1$ vs. controls, $\beta= 0.71-0.74$) (Figure 6-14) and $SMADO_2$ (at 30-60 min, $p=0.06-0.1$ vs. controls, $\beta= 0.60-0.76$) (Figure 6-15).

The RA FI and $RADO_2$ decreased significantly at the end of hypoxia in all H-R groups (vs. shams, $p<0.001$) (Figures 6-16 and 6-17). During reoxygenation, the RA FI and $RADO_2$ recovered partially (at 120 min of reoxygenation: vs. shams, $p=0.08$ and $p=0.1$, respectively). There was no significant effect on RA FI with dobutamine infusion. Apart from that 20 μg/kg/min dobutamine resulted in a transient increase in $RADO_2$ at 30 min of infusion (vs. controls, $p<0.05$), dobutamine infusion had no significant effect on $RADO_2$ (Figure 6-17).

Discussion

In this study, we have shown that high dose of dobutamine (20 μg/kg/min) had a significant inotropic effect with increased CI and SVI. The effect was intermediate when low to moderate (5-10 μg/kg/min) doses of dobutamine were used. Infusion of dobutamine did not have any significant effect on heart rate, SAP or SVRI. On the other hand, the infusion of dobutamine resulted in a significant reduction in PVRI and mild reduction in PAP and maintained the PAP below SAP (PAP/SAP ratio <1). The infusion of high dose of dobutamine also improved $sysDO_2$ with no effect on $sysVO_2$, $sysEO_2$, acid-base balance and plasma lactate concentrations. Regarding the regional hemodynamics, there was a mild but transient improvement of the SMA FI and $SMADO_2$ at 20 μg/kg/min of dobutamine infusion with no effect on the other regional circulations or oxygen deliveries.

Systemic hemodynamic effects of dobutamine

Under normal circumstances, dobutamine can increase cardiac output by increasing heart rate (chronotropy) (28), increasing stroke volume (inotropy) (25) or both. In healthy neonatal piglets, Cheung el al showed dobutamine infusion can have both effects depending on the duration of infusion (25). During the short-term (30 min) infusion of dobutamine, the increase in cardiac output was due to the chronotropic effect while it was due to the inotropic effect during the long-term (120 min) infusion. In this study, we found the increase in CI during dobutamine infusion was mainly due to the increased SVI rather than tachycardia, which happened similarly in all H-R groups. In our model of H-R induced myocardial dysfunction, the increased SVI with dobutamine infusion suggests that dobutamine was able to correct the contractile dysfunction although we did not directly measure the myocardial contractility. However, due to sample size, the chronotropic role of dobutamine infusion can not be excluded entirely. The chronotropic effect of dobutamine may not become evident in these H-R animals with tachycardia after reoxygenation (29).

The effect of dobutamine on SAP during the neonatal period is variable (25,28-30). Consistent with other reports, our findings showed that dobutamine infusion had no significant effect (increase or decrease) on the SAP. Concerns have been raised in using SAP to reflect cardiac output (31). Kluckow et al showed in preterm infants a significant but weak correlation (r=0.38) between the SAP and cardiac output (32). Therefore, using the SAP as a measure of the inotropic action of dobutamine is unreliable and a direct flow measurement is needed (32-34). In fact dobutamine has a higher failure rate than other inotropes in correcting the low SAP in critically ill preterm neonates (35), although it increases cardiac output, manifested by increased superior vena caval flow (33). Our findings show similar systemic hemodynamic effects of dobutamine in H-R piglets. Therefore, for effective increases in SAP and cardiac output in asphyxiated neonates, a vasopressor agent such as dopamine may be added to dobutamine infusion.

Pulmonary hemodynamic effect of dobutamine

Neonatal asphyxia is known to cause pulmonary hypertension with increased PVRI that were also seen in our model of H-R (19,36,37). Cheung et al found that dobutamine infusion can cause a significant increase in the PAP in normoxic piglets (25). Therefore, the authors cautioned about the possibility of exacerbating the pulmonary hypertension that may accompany perinatal asphyxia when dobutamine is being used. However, similar to the observations of Crowley et al (23), we found a significant reduction in the PVRI during the dobutamine infusion with a modest effect on the PAP in the H-R piglets. Furthermore, despite no significant effect on the SAP or SVRI during dobutamine infusion, the PAP/SAP ratio was maintained at a ratio of <1 whereas the ratio in controls was ≥1 suggesting suprasystemic PAP. This finding may be important in the choice of inotropes to treat post-asphyxial myocardial dysfunction in the critically ill neonates (31). In this scenario, dobutamine would enhance the cardiac output without exacerbating the pulmonary hypertension. In fact, it may even decrease the right-to-left shunting at the ductal level by maintaining a lower PAP/SAP ratio. Other inotropes have been shown to either exacerbate the effect of H-R and asphyxia on the pulmonary vasculature (14,38,39) which can worsen the pulmonary hypertension and right-to-left shunting.

Systemic oxygen metabolism during dobutamine infusion

There is a concern of increased sysVO_2 when dobutamine is being used (40,41). In healthy newborn lambs (1-2 day old), Penny el al found that dobutamine infusion was associated with a substantial increase in the sysVO_2 which accounted for approximately 90% of the sysDO_2 (42). In contrast to the study by Penny et al, we did not observe any significant changes in sysVO_2 or sysEO_2 that accompanied the significantly increased sysDO_2 during dobutamine infusion. The negative results may be related to the small sample size. However, hypoxia and reoxygenation can lead to cellular dysfunction which may diminish oxygen consumption by inhibiting mitochondrial respiration (43,44). Species difference may also account for the different observations. Interestingly, neonatal piglets are lacking the mitochondrial uncoupling protein and do not have brown adipose tissue (45) while neonatal lambs do have this protein (46). This protein is probably responsible for the non-shivering heat generation (47) and the increase in sysVO_2 in neonatal lambs (48). The generalization of these findings in the human neonates requires caution. This is because human neonates have brown adipose tissue and mitochondrial uncoupling protein but to a lesser extent than that of neonatal lambs (49).

With improvement in sysDO_2 we did not observe any significant increase in the plasma lactate concentration in any of the dobutamine-treated groups. This is probably due to the absence of systemic vasoconstriction and secondary tissue hypoxia that may be seen with other inotropes (17) or vasopressors (50).

Regional hemodynamic effects of dobutamine

Apart from the mild and transient increase in SMA FI and $SMADO_2$, dobutamine infusion did not result in any significant effect on the regional circulation or oxygen delivery. Cheung et al showed no effect of dobutamine infusion on mesenteric or renal circulations in doses 5-50 μg/kg/min (25) in healthy neonatal piglets. Both SMA FI and RA FI increased significantly when dobutamine was infused at a dose of 10 μg/kg/min for a longer period of time (2h). In the current study, we studied the effect of dobutamine infusion over a similar duration. However, we used sick animals with multiorgan dysfunction and regional circulatory deficit as evident by other studies using similar models (29,51,52). We speculate that the regional effect of dobutamine might have been blunted in H-R. Moreover, the duration of infusion was probably not long enough to observe an effect on the regional circulation.

Clinical implications

Based on our findings in this model of H-R, dobutamine is an appropriate inotrope to treat post-asphyxial myocardial dysfunction. This can be achieved without adversely affecting the regional circulation or $sysVO_2$. Using dobutamine under these circumstances may also attenuate the effect of asphyxia on pulmonary vascular resistance. Dobutamine infusion would decrease the PVRI and minimize the shunting across the ductus arteriosus. Therefore, dobutamine may help in the treatment of pulmonary hypertension of the newborn that may result from neonatal asphyxia.

In asphyxiated neonates, treatment is usually started to treat low SAP but dobutamine does not increase the SAP effectively. The combination of dobutamine with a vasopressor such as dopamine (5 μg/kg/min) might be a therapeutic option. Low dose dopamine may increase the SAP due to its vasopressor effect which in combination of dobutamine can enhance the blood flow (22,33,35,53). To our knowledge, there is no study that examined this combination. Furthermore, the prolonged infusion and the use of very high dose (40 μg/kg/min) of dobutamine during reoxygenation after hypoxia need to be studied.

Conclusions

In newborn piglets with hypoxia, myocardial stunning with low cardiac output and hypotension gradually developed during reoxygenation. High dose dobutamine exerts an inotropic action without any effect on blood pressure. Dobutamine infusion maintained a lowered pulmonary vascular resistance and did not have an adverse effect on the regional circulations or oxygen transport.

Table 6-1: Acid-base balance in newborn piglets during hypoxia, reoxygenation and dobutamine infusion. Control piglets received saline infusion. (*n*=8 in each group).

	Control	Dobutamine (μg/kg/min)		
		5	10	20
Baseline				
pH	7.43±0.04	7.39±0.03	7.36±0.03*	7.36±0.04*
PaO_2 (mmHg)	71±14	65±6	70±10	70±8
HCO_3^- (mmol/L)	23±3	22±3	23±3	24±2
2h Hypoxia				
pH	7.05±0.09	7.02±0.16	7.03±0.07	7.06±0.09
PaO_2 (mmHg)	31±8	33±6	36±7	31±8
HCO_3^- (mmol/L)	11±3	9±4	9±3	11±3
10min Reoxygenation				
pH	7.05±0.10	7.00±0.20	7.01±0.11	7.10±0.10
PaO_2 (mmHg)	509±42	491±70	488±61	456±61
HCO_3^- (mmol/L)	10±3	8±4	9±2	11±3
2h Reoxygenation				
pH	7.35±0.05	7.34±0.05	7.32±0.04	7.34±0.08
PaO_2 (mmHg)	62±12	69±11	64±8	63±8
HCO_3^- (mmol/L)	21±2	23±3	20±2	21±3
2h Dobutamine or saline infusion				
pH	7.32±0.06	7.32±0.07	7.30±0.05	7.34±0.08
PaO_2 (mmHg)	61±9	66±12	63±9	59±4
HCO_3^- (mmol/L)	20±3	20±3	18±3	21±3

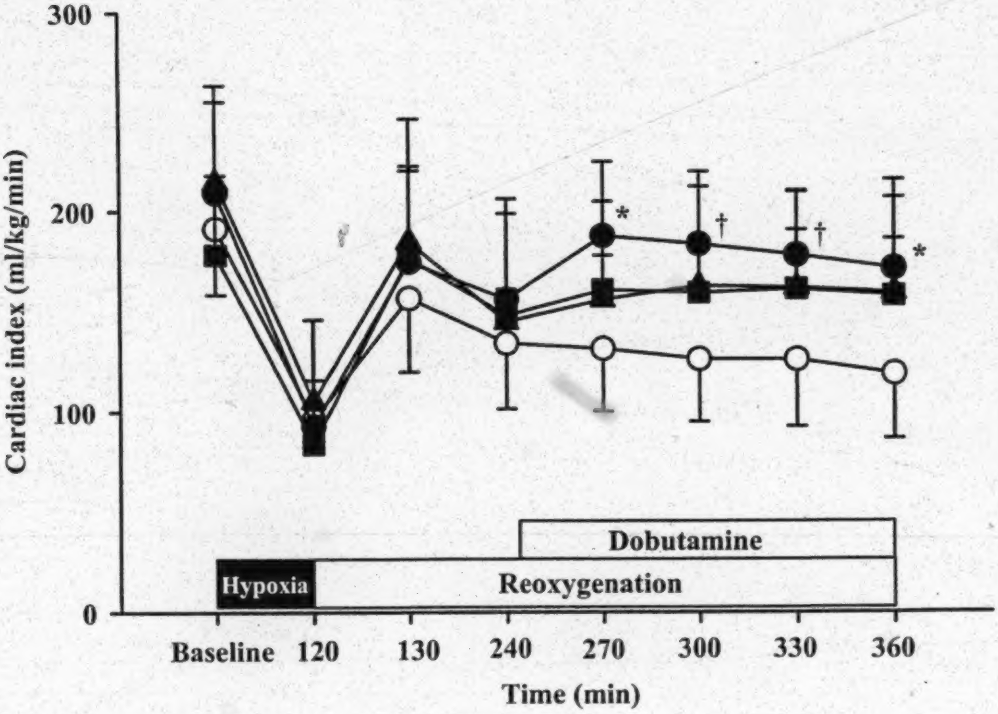
Values are mean±SD. *p<0.05 vs. control, ANOVA

Table 6-2: Plasma lactate (mmol/L) in newborn piglets during hypoxia, reoxygenation and dobutamine infusion. Control piglets received saline infusion. (*n*=8 in each group).

	Control	Dobutamine (µg/kg/min)		
		5	10	20
Baseline	4.8±1.6	5.8±1.2	5.3±1.7	4.8±1.4
2h Hypoxia	14.5±1.4	15.0±1.9	14.4±2.5	14.1±1.8
2h Reoxygenation	6.5±1.7	7.0±2.9	6.7±3.6	6.2±3.0
2h Dobutamine or saline infusion	5.3±2.5	5.8±2.2	5.7±4.4	5.1±2.6

Values are mean±SD.

Figure 6-1: The effect of dobutamine infusion in cardiac index in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

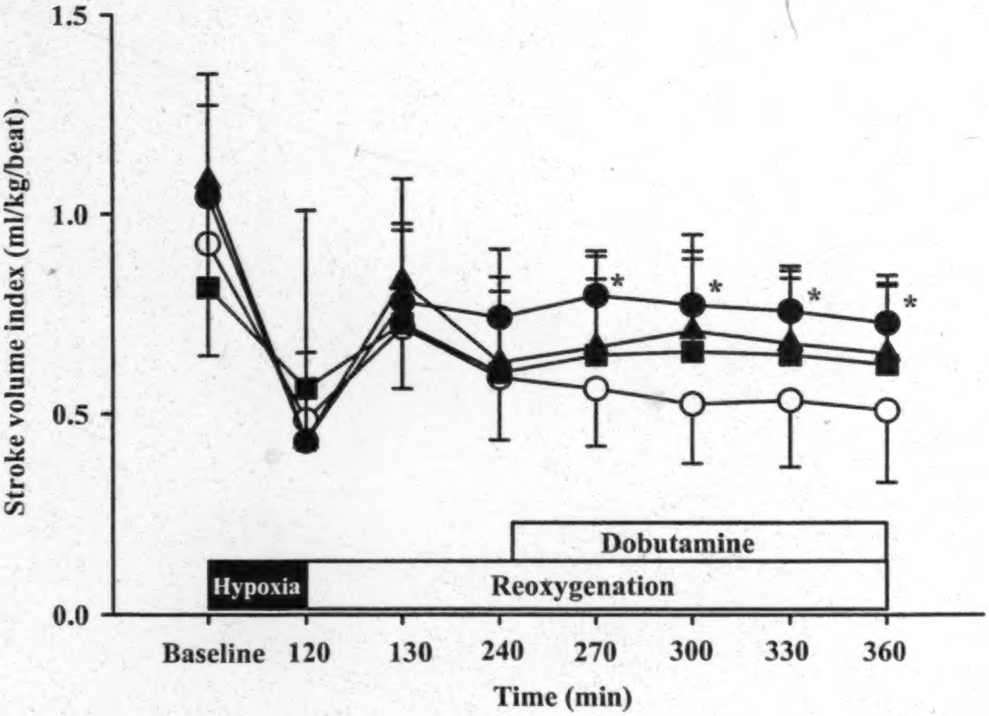
▲ Dobutamine 5 µg/kg/min (*n*=8)

■ Dobutamine 10 µg/kg/min (*n*=8)

● Dobutamine 20 µg/kg/min (*n*=8)

* p<0.05 and † p=0.06-0.08 vs. control

Figure 6-2: The effect of dobutamine infusion in stroke volume index in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

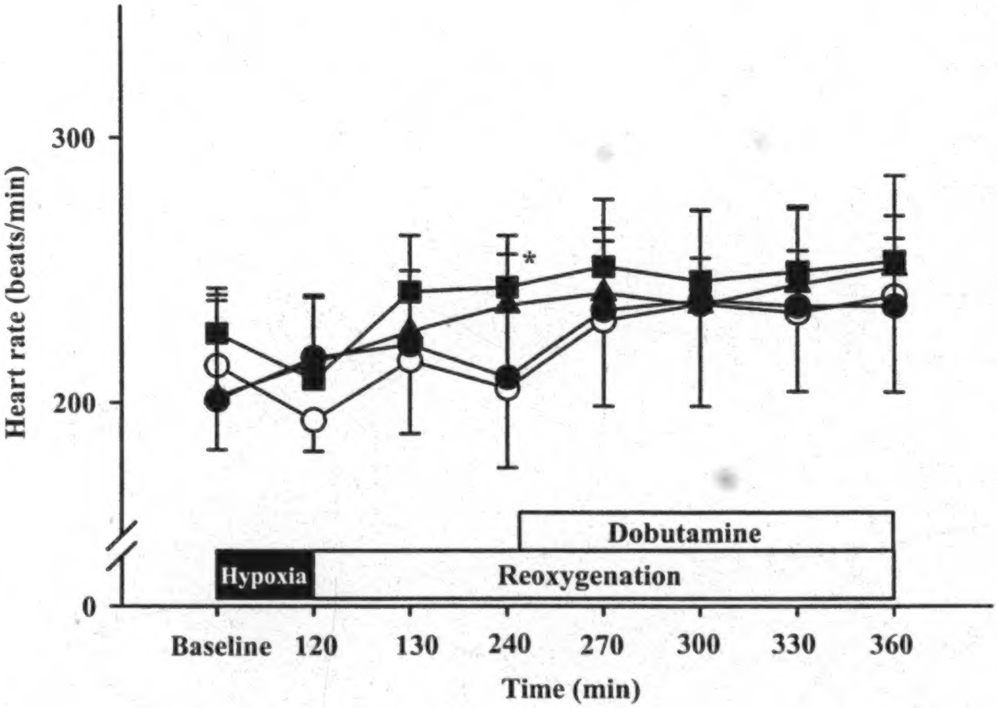
▲ Dobutamine 5 μg/kg/min (*n*=8)

■ Dobutamine 10 μg/kg/min (*n*=8)

● Dobutamine 20 μg/kg/min (*n*=8)

* $p<0.05$ vs. control

Figure 6-3: The effect of dobutamine infusion in heart rate in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

▲ Dobutamine 5 µg/kg/min (*n*=8)

■ Dobutamine 10 µg/kg/min (*n*=8)

● Dobutamine 20 µg/kg/min (*n*=8)

* $p<0.05$ vs. control

Figure 6-4: The effect of dobutamine infusion in mean systemic arterial pressure in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.

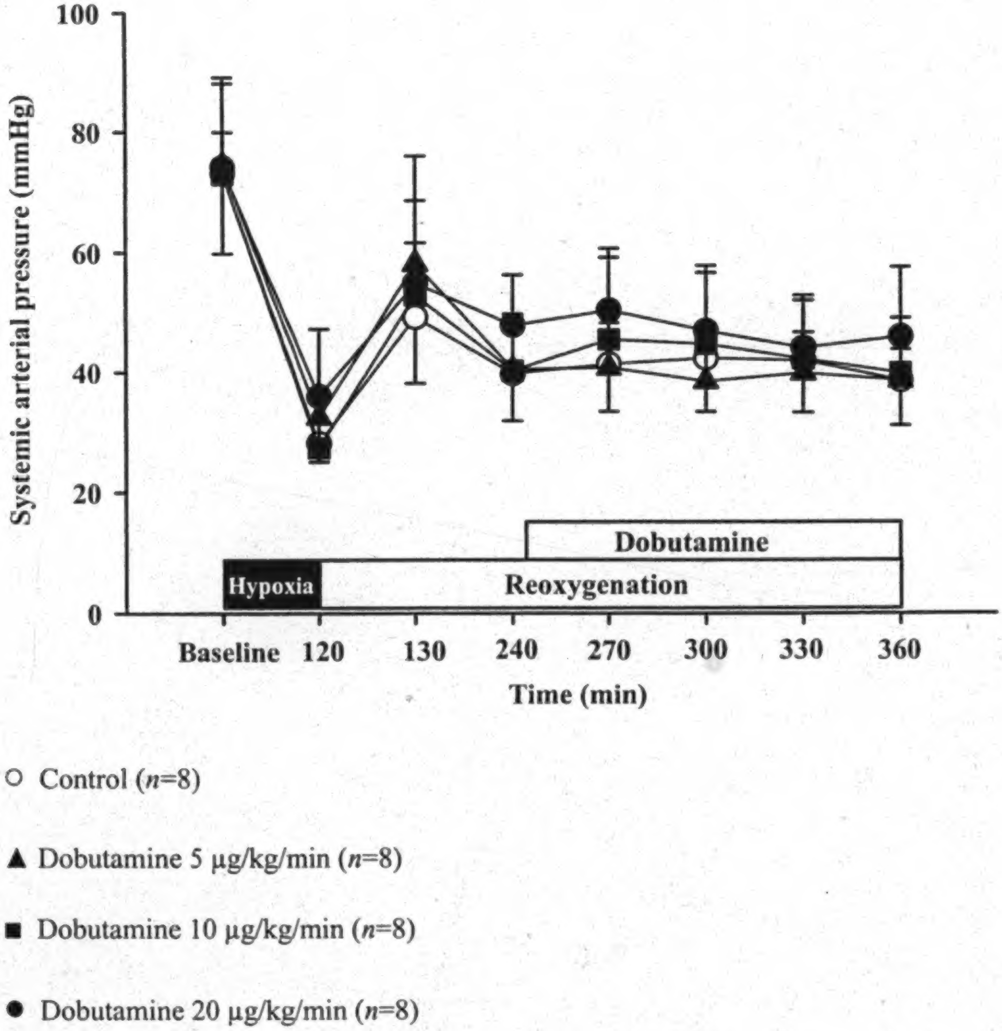
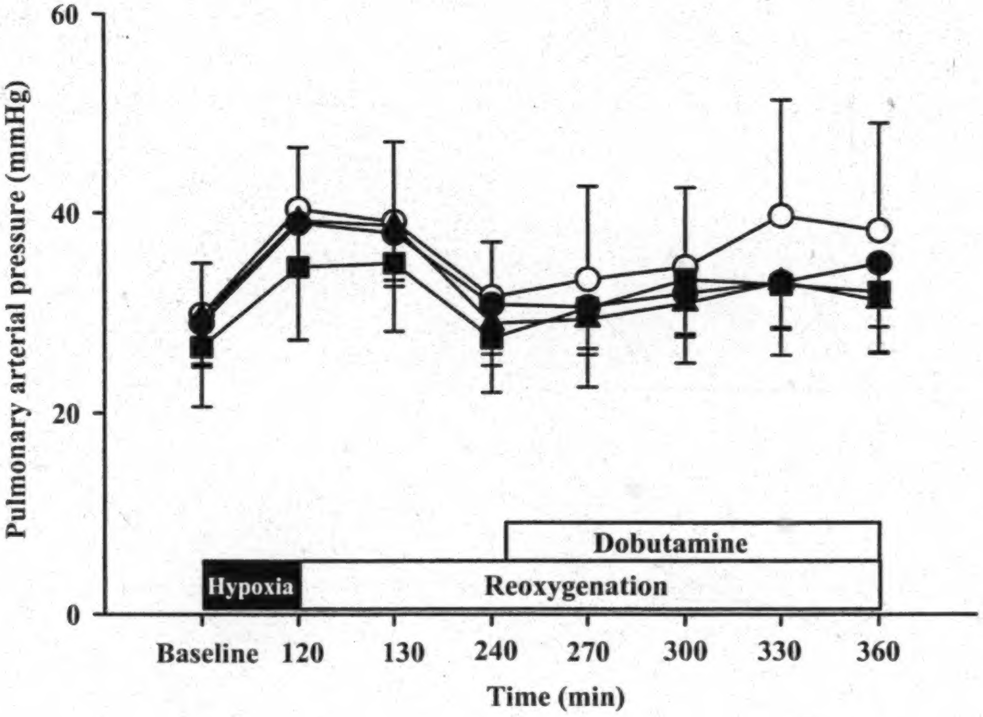


Figure 6-5: The effect of dobutamine infusion in mean pulmonary arterial pressure in newborn piglets during hypoxia reoxygenation. Error bars represent standard deviation.



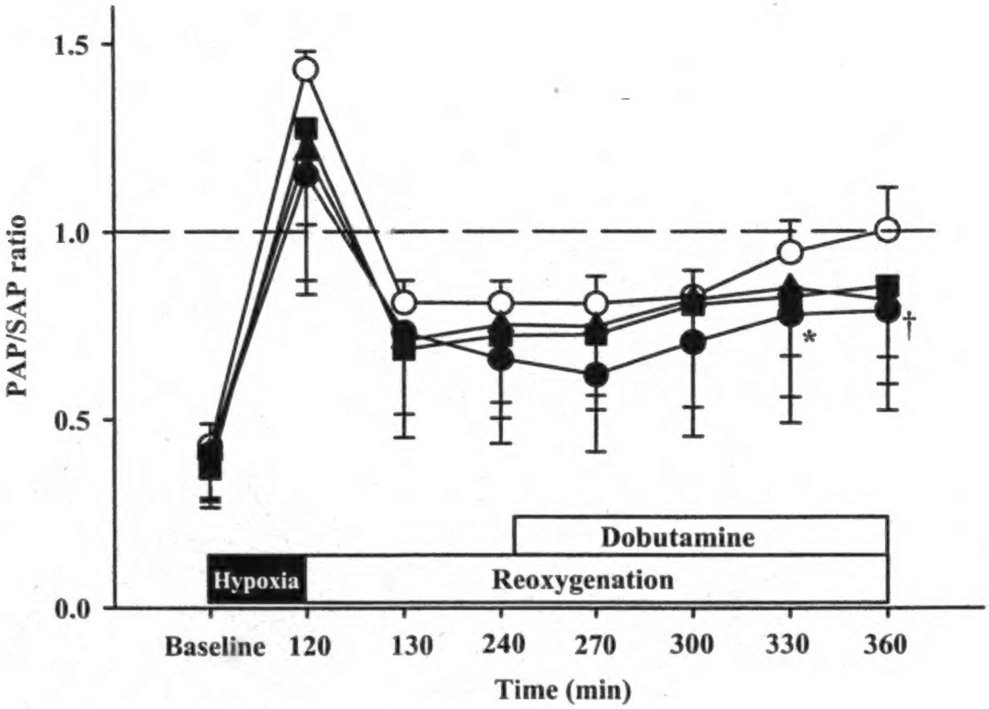
○ Control (*n*=8)

▲ Dobutamine 5 µg/kg/min (*n*=8)

■ Dobutamine 10 µg/kg/min (*n*=8)

● Dobutamine 20 µg/kg/min (*n*=8)

Figure 6-6: The effect of dobutamine infusion in pulmonary arterial pressure/systemic arterial pressure (PAP/SAP) ratio in newborn piglets during hypoxia reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

▲ Dobutamine 5 μg/kg/min (*n*=8)

■ Dobutamine 10 μg/kg/min (*n*=8)

● Dobutamine 20 μg/kg/min (*n*=8)

* $p<0.05$ and † $p=0.08$ vs. control, ANOVA

Dashed-line represents the value above which the PAP is suprasystemic and may lead to increased right-to-left shunting across the ductus arteriosus.

Figure 6-7: The effect of dobutamine infusion in systemic vascular resistance index (SVRI) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.

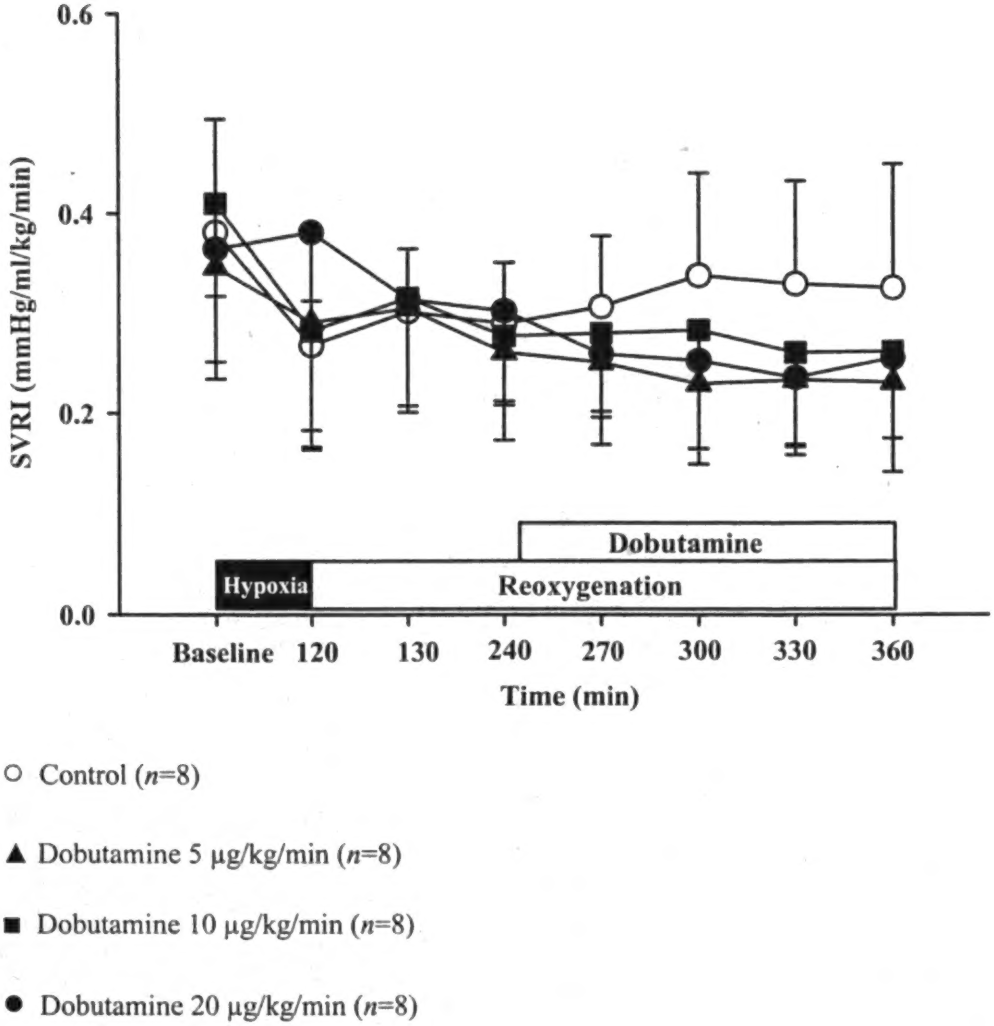
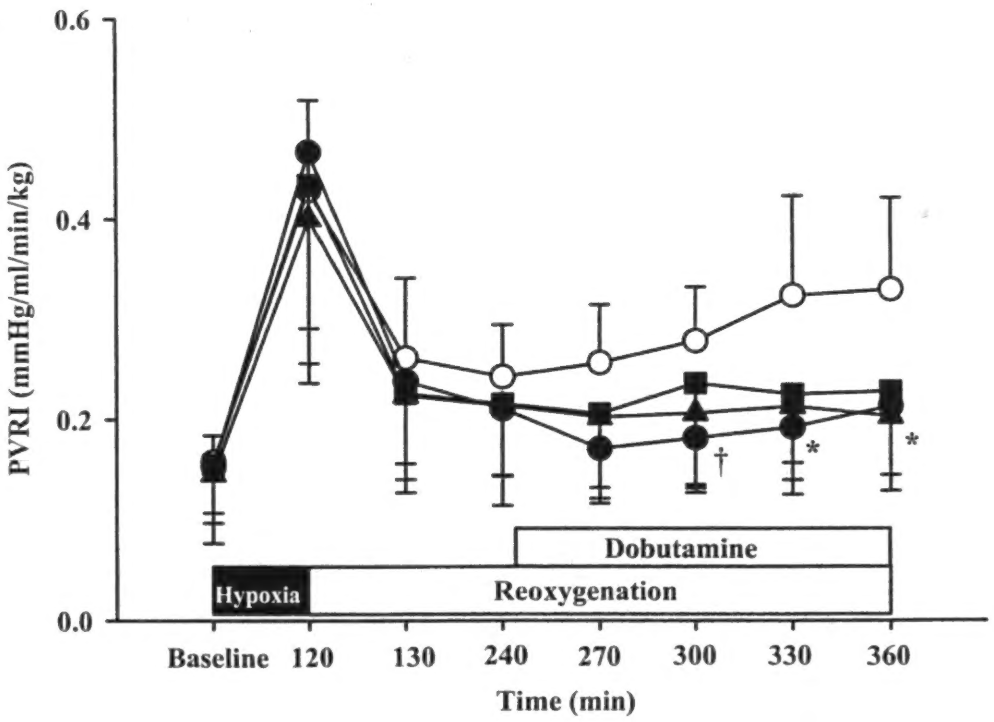


Figure 6-8: The effect of dobutamine infusion in estimated pulmonary vascular resistance index (PVRI) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

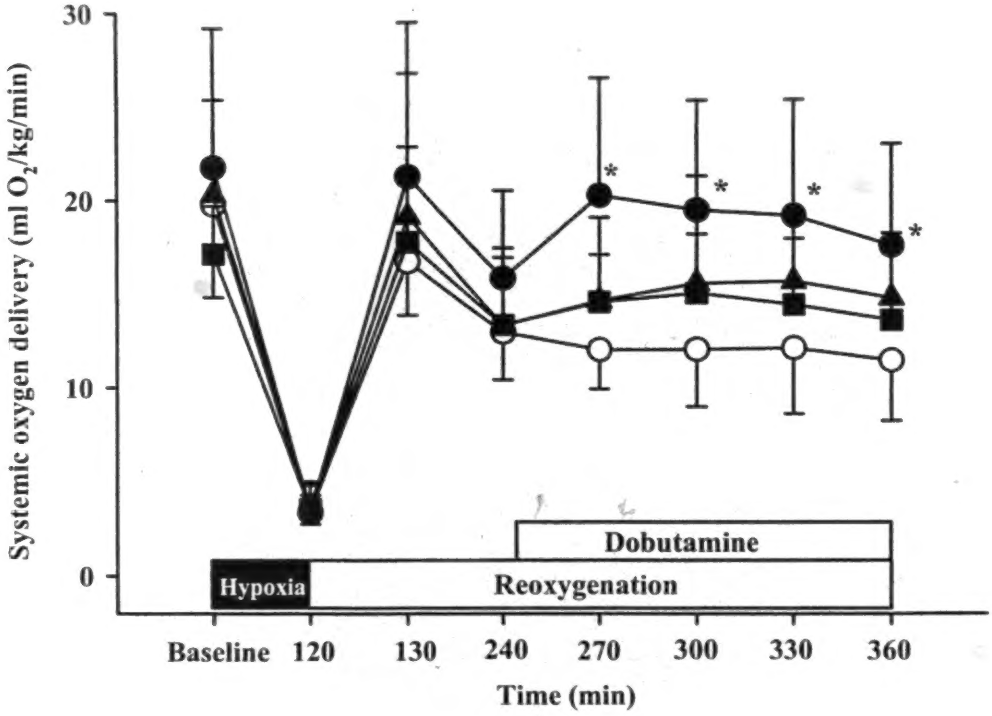
▲ Dobutamine 5 µg/kg/min (*n*=8)

■ Dobutamine 10 µg/kg/min (*n*=8)

● Dobutamine 20 µg/kg/min (*n*=8)

* p<0.05 and † p=0.06 vs. control

Figure 6-9: The effect of dobutamine infusion in systemic oxygen delivery in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

▲ Dobutamine 5 μg/kg/min (*n*=8)

■ Dobutamine 10 μg/kg/min (*n*=8)

● Dobutamine 20 μg/kg/min (*n*=8)

* $p<0.05$ vs. control

Figure 6-10: The effect of dobutamine infusion in systemic oxygen consumption in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.

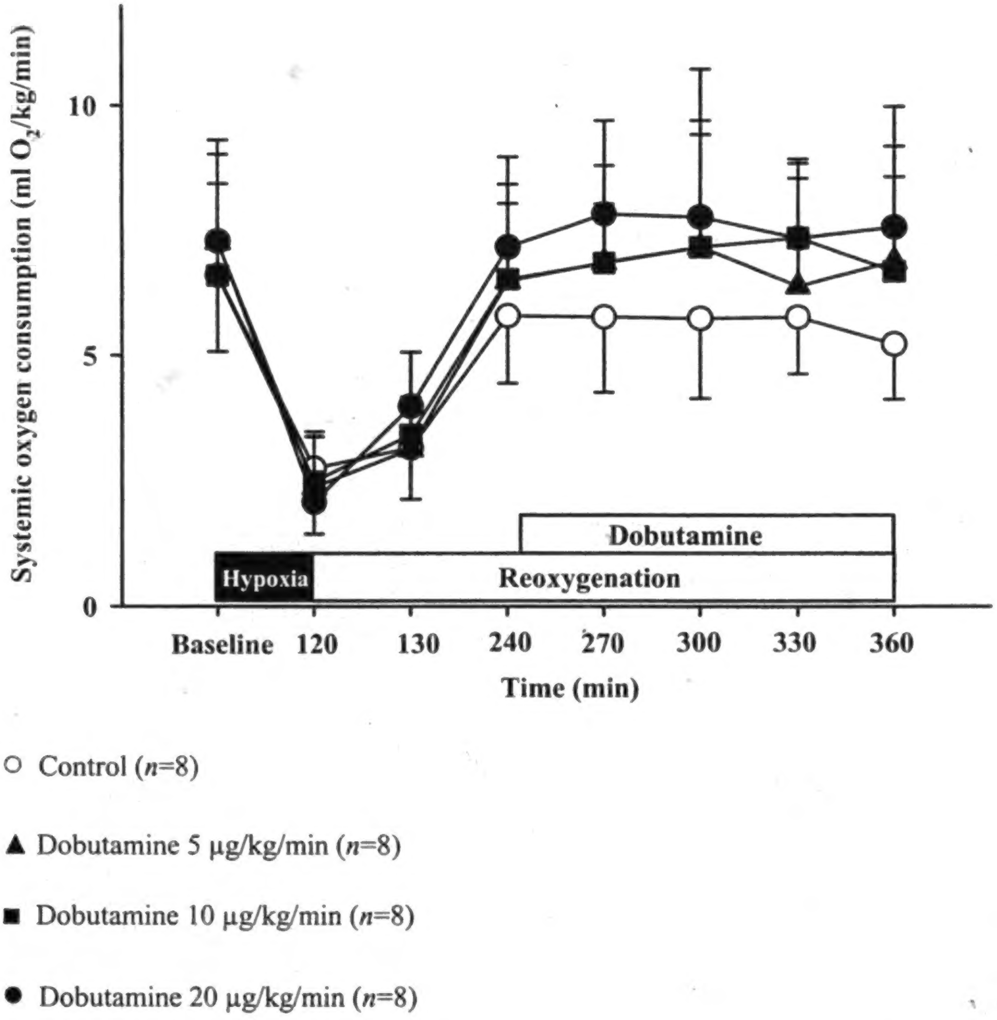
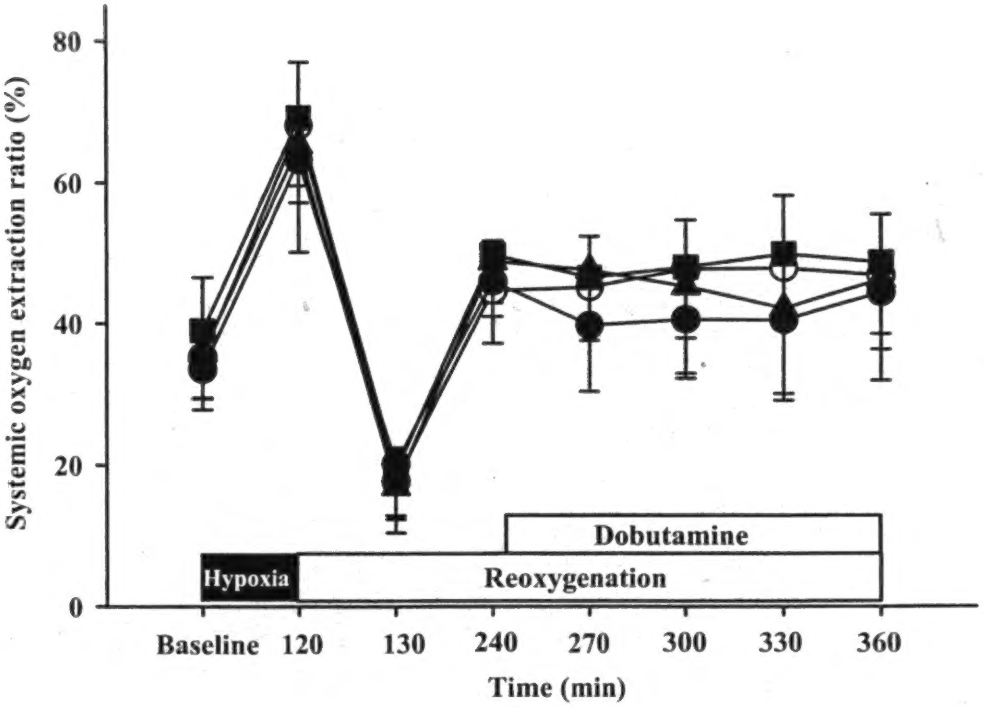


Figure 6-11: The effect of dobutamine infusion in systemic oxygen extraction ratio in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



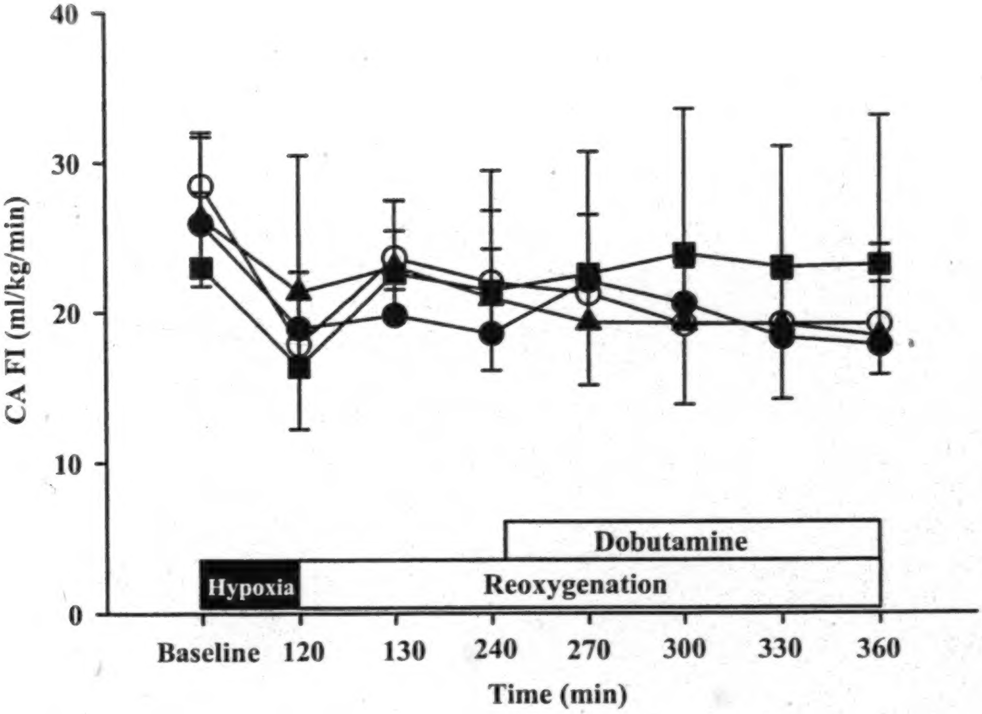
○ Control (*n*=8)

▲ Dobutamine 5 µg/kg/min (*n*=8)

■ Dobutamine 10 µg/kg/min (*n*=8)

● Dobutamine 20 µg/kg/min (*n*=8)

Figure 6-12: The effect of dobutamine infusion in carotid arterial flow index (CA FI) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

▲ Dobutamine 5 μg/kg/min (*n*=8)

■ Dobutamine 10 μg/kg/min (*n*=8)

● Dobutamine 20 μg/kg/min (*n*=8)

Figure 6-13: The effect of dobutamine infusion in carotid arterial oxygen delivery ($CADO_2$) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.

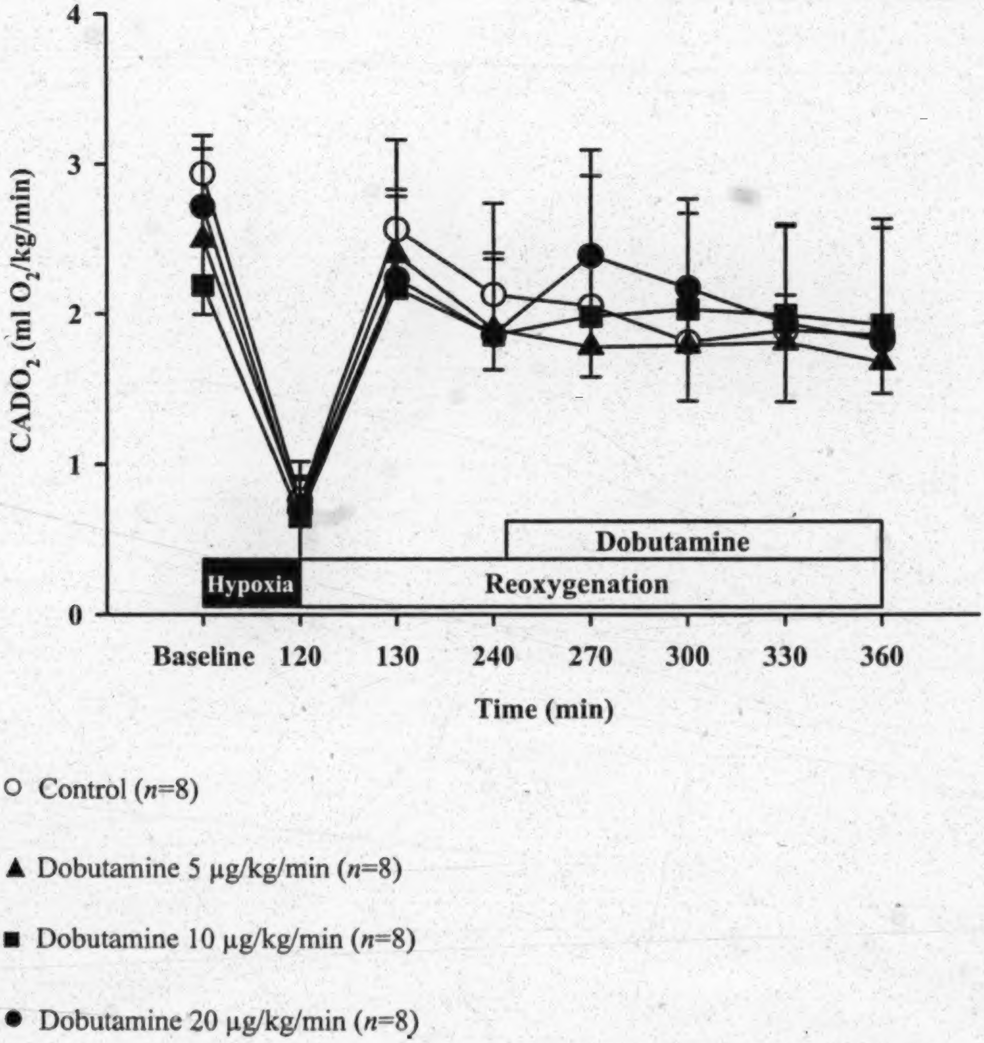
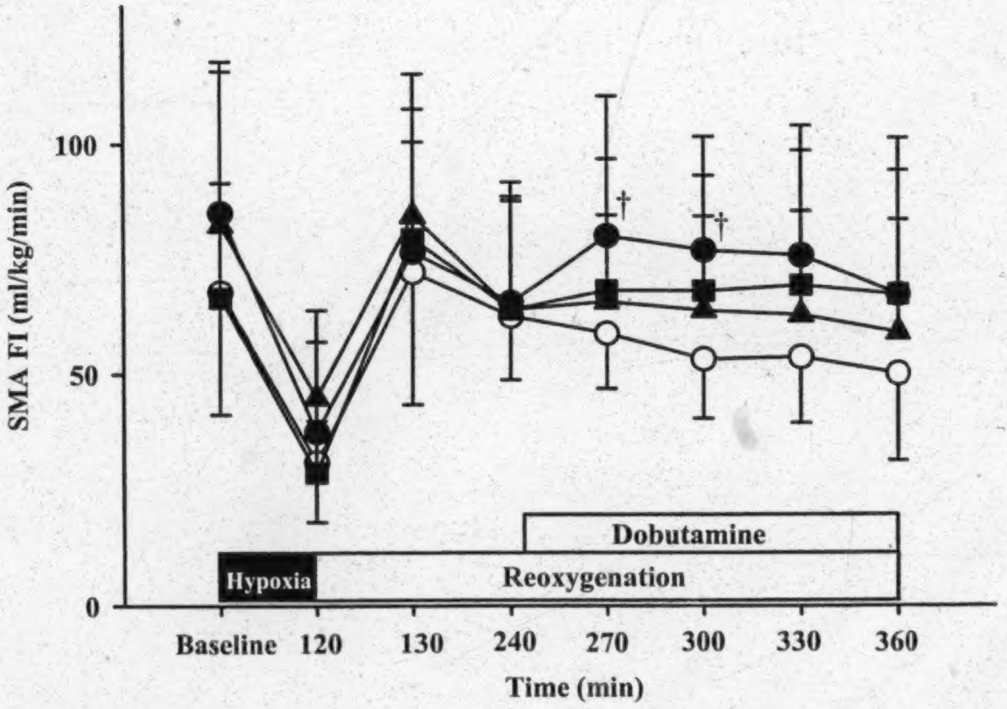


Figure 6-14: The effect of dobutamine infusion in superior mesenteric arterial flow index (SMA FI) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

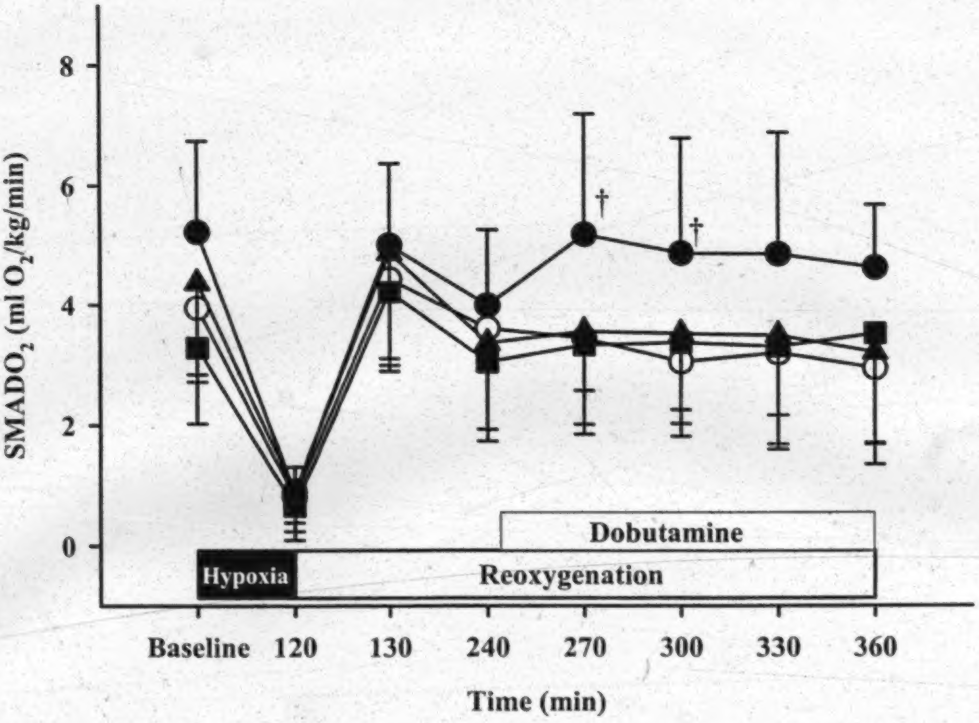
▲ Dobutamine 5 µg/kg/min (*n*=8)

■ Dobutamine 10 µg/kg/min (*n*=8)

● Dobutamine 20 µg/kg/min (*n*=8)

† p=0.1 vs. controls

Figure 6-15: The effect of dobutamine infusion in superior mesenteric arterial oxygen delivery ($SMADO_2$) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

▲ Dobutamine 5 μg/kg/min (*n*=8)

■ Dobutamine 10 μg/kg/min (*n*=8)

● Dobutamine 20 μg/kg/min (*n*=8)

† p=0.06-0.1 vs. controls

Figure 6-16: The effect of dobutamine infusion in renal arterial flow index (RA FI) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.

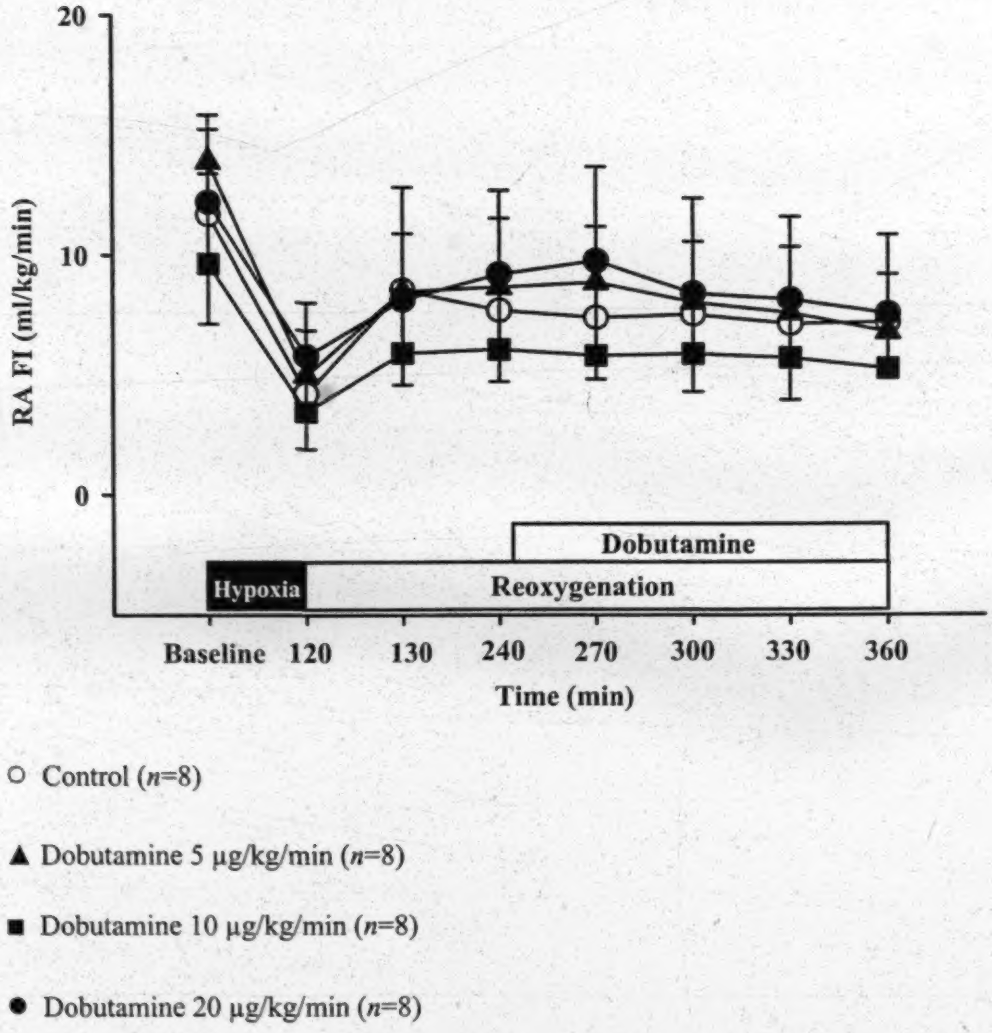
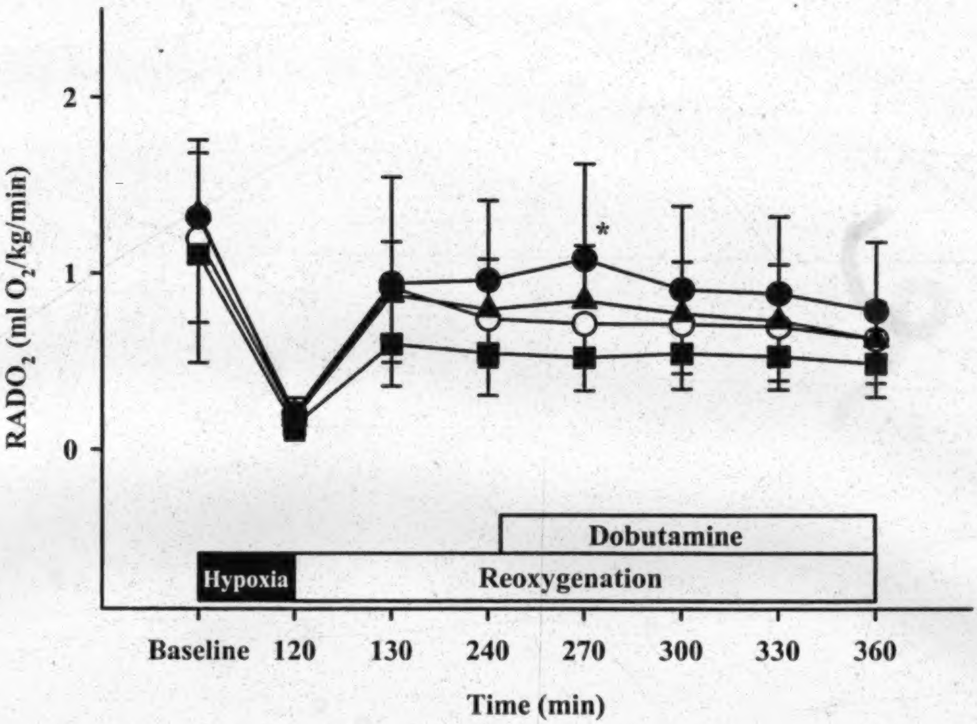


Figure 6-17: The effect of dobutamine infusion in renal arterial oxygen delivery ($RADO_2$) in newborn piglets during hypoxia and reoxygenation. Error bars represent standard deviation.



○ Control (*n*=8)

▲ Dobutamine 5 μg/kg/min (*n*=8)

■ Dobutamine 10 μg/kg/min (*n*=8)

● Dobutamine 20 μg/kg/min (*n*=8)

* $p<0.05$ vs. control

References

1. Martin-Ancel A, Garcia-Alix A, Gaya F, Cabanas F, Burgueros M, Quero J 1995 Multiple organ involvement in perinatal asphyxia. J Pediatr 127:786-793
2. World Health Organization 2005 The World Health Report 2005. Geneva
3. Anderson RN 2002 Deaths: leading causes for 2000. Natl Vital Stat Rep 50:1-85
4. Robertson CM, Finer NN, Grace MG 1989 School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. J Pediatr 114:753-760
5. Cabal LA, Devaskar U, Siassi B, Hodgman JE, Emmanouilides G 1980 Cardiogenic shock associated with perinatal asphyxia in preterm infants. J Pediatr 96:705-710
6. Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB 1988 Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest 82:476-485
7. Li C, Jackson RM 2002 Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol 282:C227-C241
8. Zweier JL 1988 Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury. J Biol Chem 263;1353-1357
9. Ranjit MS 2000 Cardiac abnormalities in birth asphyxia. Indian J Pediatr 67:S26-S29
10. Piazza AJ 1999 Postasphyxial management of the newborn. Clin Perinatol 26:749-65

11. Seri I, Evans J 2001 Controversies in the diagnosis and management of hypotension in the newborn infant. Curr Opin Pediatr 13:116-123

12. Osborn DA, Evans N 2001 Early volume expansion versus inotrope for prevention of morbidity and mortality in very preterm infants. Cochrane Database Syst RevCD002056

13. Subhedar NV, Shaw NJ 2003 Dopamine versus dobutamine for hypotensive preterm infants. Cochrane Database Syst RevCD001242

14. Cheung PY, Barrington KJ, Pearson RJ, Bigam DL, Finer NN, Van Aerde JE 1997 Systemic, pulmonary and mesenteric perfusion and oxygenation effects of dopamine and epinephrine. Am J Respir Crit Care Med 155:32-37

15. Zhang J, Penny DJ, Kim NS, Yu VYH, Smolich JJ 1999 Mechanisms of blood pressure increase induced by dopamine in hypotensive preterm neonates. Arch Dis Child Fetal Neonatal Ed 81:F99-104

16. Roze JC, Tohier C, Maingueneau C, Lefevre M, Mouzard A 1993 Response to dobutamine and dopamine in the hypotensive very preterm infant. Arch Dis Child 69:59-63

17. Valverde E, Pellicer A, Madero R, Elorza D, Quero J, Cabanas F 2006 Dopamine Versus Epinephrine for Cardiovascular Support in Low Birth Weight Infants: Analysis of Systemic Effects and Neonatal Clinical Outcomes. Pediatrics 117:e1213-e1222

18. Paradisis M, Osborn DA 2004 Adrenaline for prevention of morbidity and mortality in preterm infants with cardiovascular compromise. Cochrane Database Syst RevCD003958

19. Bigam DL, Barrington KJ, Jirsch DW, Cheung PY 1998 Effects of a continuous epinephrine infusion on regional blood flow in awake newborn piglets. Biol Neonate 73:198-206

20. Cheung PY, Barrington KJ 2001 The effects of dopamine and epinephrine on hemodynamics and oxygen metabolism in hypoxic anesthetized piglets. Crit Care 5:158-166

21. Valverde E, Pellicer A, Madero R, Elorza D, Quero J, Cabanas F 2006 Dopamine Versus Epinephrine for Cardiovascular Support in Low Birth Weight Infants: Analysis of Systemic Effects and Neonatal Clinical Outcomes. Pediatrics 117:e1213-e1222

22. Ruffolo RR, Jr., Messick K 1985 Effects of dopamine, (+/-)-dobutamine and the (+)- and (-)-enantiomers of dobutamine on cardiac function in pithed rats. J Pharmacol Exp Ther 235:558-565

23. Crowley MR, Fineman JR, Soifer SJ 1991 Effects of vasoactive drugs on thromboxane A2 mimetic-induced pulmonary hypertension in newborn lambs. Pediatr Res 29:167-172

24. Ferrara JJ, Dyess DL, Peeples GL, Christenberry DP, Roberts WS, Tacchi EJ, Swafford AN, Ardell JL, Powell RW 1995 Effects of dopamine and dobutamine on regional blood flow distribution in the neonatal piglet. Ann Surg 221:531-540

25. Cheung PY, Barrington KJ, Bigam D 1999 The hemodynamic effects of dobutamine infusion in the chronically instrumented newborn piglet. Crit Care Med 27:558-564

26. Cheung PY, Stevens JP, Haase E, Stang L, Bigam DL, Etches W, Radomski MW 2006 Platelet dysfunction in asphyxiated newborn piglets resuscitated with 21% and 100% oxygen. Pediatr Res 59:636-640

27. Passonneau JV, Lowry 0H 1993 Enzymatic analysis: A practical guide. Humana Press, Totowa NJ

28. Fiser DH, Fewell JE, Hill DE, Brown AL 1988 Cardiovascular and renal effects of dopamine and dobutamine in healthy, conscious piglets. Crit Care Med 16:340-345

29. Haase E, Bigam DL, Nakonechny QB, Rayner D, Korbutt G, Cheung PY 2005 Cardiac function, myocardial glutathione, and matrix metalloproteinase-2 levels in hypoxic newborn pigs reoxygenated by 21%, 50%, or 100% oxygen. Shock 23:383-389

30. Nudel DB, Peterson BJ, Buckley BJ, Gootman N, Hoffman RG 1991 Effects of acute hypoxemia on responses to dopamine and dobutamine in neonatal swine. Methods Find Exp Clin Pharmacol 13:111-119

31. Evans N 2006 Which inotrope for which baby? Arch Dis Child Fetal Neonatal Ed 91:F213-F220

32. Kluckow M, Evans N 1996 Relationship between blood pressure and cardiac output in preterm infants requiring mechanical ventilation. J Pediatr 129:506-512

33. Osborn D, Evans N, Kluckow M 2002 Randomized trial of dobutamine versus dopamine in preterm infants with low systemic blood flow. J Pediatr 140:183-191

34. Kluckow M, Evans N 2000 Superior vena cava flow in newborn infants: a novel marker of systemic blood flow. Arch Dis Child Fetal Neonatal Ed 82:F182-F187

35. Klarr JM, Faix RG, Pryce CJ, Bhatt-Mehta V 1994 Randomized, blind trial of dopamine versus dobutamine for treatment of hypotension in preterm infants with respiratory distress syndrome. J Pediatr 125:117-122

36. Medbo S, Yu XQ, Asberg A, Saugstad OD 1998 Pulmonary hemodynamics and plasma endothelin-1 during hypoxemia and reoxygenation with room air or 100% oxygen in a piglet model. Pediatr Res 44:843-849

37. Dakshinamurti S 2005 Pathophysiologic mechanisms of persistent pulmonary hypertension of the newborn. Pediatr Pulmonol 39:492-503

38. Liet JM, Boscher C, Gras-Leguen C, Gournay V, Debillon T, Roze JC 2002 Dopamine effects on pulmonary artery pressure in hypotensive preterm infants with patent ductus arteriosus. J Pediatr 140:373-375

39. Phillipos EZ, Robertson MA 2000 Abstract randomized double blinded controlled trial of dopamine (DO) versus epinephrine (EPI) on pulmonary artery pressure (PAP) in premature infants < 1750 grams. Pediatr Res 47:425Abstact

40. Bhatt SB, Hutchinson RC, Tomlinson B, Oh TE, Mak M 1992 Effect of dobutamine on oxygen supply and uptake in healthy volunteers. Br J Anaesth 69:298-303

41. Abdul-Rasool IH, Chamberlain JH, Swan PC, Ward DS 1987 Cardiorespiratory and metabolic effects of dopamine and dobutamine infusions in dogs. Crit Care Med 15:1044-1050

42. Penny DJ, Sano T, Smolich JJ 2001 Increased systemic oxygen consumption offsets improved oxygen delivery during dobutamine infusion in newborn lambs. Intensive Care Med 27:1518-1525

43. Cheung PY, Danial H, Jong J, Schulz R 1998 Thiols protect the inhibition of myocardial aconitase by peroxynitrite. Arch Biochem Biophys 350:104-108

44. Szabo C, Salzman AL 1995 Endogenous peroxynitrite is involved in the inhibition of mitochondrial respiration in immuno-stimulated J774.2 macrophages. Biochem Biophys Res Commun 209:739-743

45. Trayhurn P, Temple NJ, Van AJ 1989 Evidence from immunoblotting studies on uncoupling protein that brown adipose tissue is not present in the domestic pig. Can J Physiol Pharmacol 67:1480-1485

46. Nedergaard J, Connolly E, Cannon B 1986 Brown adipose tissue in the mammalian neonate. Edward Arnold, London, pp 153-213

47. Garlid KD, Jaburek M, Jezek P, Varecha M 2000 How do uncoupling proteins uncouple? Biochim Biophys Acta 1459:383-389

48. Sihra TS, Scott IG, Nicholls DG 1984 Ionophore A23187, verapamil, protonophores, and veratridine influence the release of gamma-aminobutyric acid from synaptosomes by modulation of the plasma membrane potential rather than the cytosolic calcium. J Neurochem 43:1624-1630

49. Lean ME, James WP, Jennings G, Trayhurn P 1986 Brown adipose tissue uncoupling protein content in human infants, children and adults. Clin Sci (Lond) 71:291-297

50. Day NP, Phu NH, Bethell DP, Mai NT, Chau TT, Hien TT, White NJ 1996 The effects of dopamine and adrenaline infusions on acid-base balance and systemic haemodynamics in severe infection. Lancet 348:219-223

51. Haase E, Bigam DL, Nakonechny QB, Jewell LD, Korbutt G, Cheung PY 2004 Resuscitation with 100% oxygen causes intestinal glutathione oxidation and reoxygenation injury in asphyxiated newborn piglets. Ann Surg 240:364-373

52. Stevens J, Haase E, Bigam DL, Cheung PY 2003 Hemodynamic changes and oxygen metabolism of the neonatal liver during hypoxia and reoxygenation with 21% and 100% oxygen. Pediatr Res 53:375Abstract

53. Hentschel R, Hensel D, Brune T, Rabe H, Jorch G 1995 Impact on blood pressure and intestinal perfusion of dobutamine or dopamine in hypotensive preterm infants. Biol Neonate 68:318-324

CHAPTER 7

The effect of dobutamine on platelet aggregatory function in newborn piglets with hypoxia and reoxygenation

Abstract

Background: Dobutamine, a β-adrenoceptor agonist that is often used to treat myocardial dysfunction in asphyxiated neonates, may act on the β-adrenoceptors on platelets resulting in activation. Little information is available on the effect of dobutamine on the platelet aggregatory function in neonatal asphyxia. Therefore, we tested the hypothesis that dobutamine used during reoxygenation after hypoxia in newborn piglets can cause platelet activation and aggregatory dysfunction.

Methods: Newborn piglets were acutely instrumented and exposed to hypoxia for 2h and reoxygenation for 4h. Piglets were randomized to receive dobutamine infusion (5, 10 or 20 μg/kg/min) or saline (control) at 2h-4h of reoxygenation (n=8 each). Sham-operated animals were not exposed to hypoxia and reoxygenation (n=6) were included. Platelet number, collagen-stimulated whole blood aggregation, plasma concentrations of thromboxane B_2 (TxB_2), cAMP, cGMP and nitrotyrosine, and plasma matrix metalloproteinase (MMP)-2 and -9 activities were studied.

Results: At the end of hypoxia, shock and metabolic acidosis developed similarly in all hypoxia-reoxygenated groups. At 4h of reoxygenation, platelet counts in all groups decreased similarly (323±90 vs. 213±87 x 10^9/L of baseline, $p<0.05$). Platelet aggregation deteriorated significantly with a rightward shift of concentration-response curve in piglets receiving 10 and 20 μg/kg/min dobutamine compared to their respective baselines. The group of 20 μg/kg/min of dobutamine had higher plasma TxB_2 compared to baseline ($p<0.05$). Hypoxia caused a significant increase in the plasma MMP-9 activity that returned back to baseline with reoxygenation. There were no significant differences

in the plasma concentrations of cAMP, cGMP or nitrotyrosine or MMP-2 activities between groups and over time.

Conclusions: The use of dobutamine at high doses for 2h tended to cause platelet activation and aggregatory dysfunction during reoxygenation of hypoxic newborn piglets.

Introduction

Asphyxia is a significant cause of morbidity and mortality in critically ill neonates (1). Along with the multi-organ dysfunction (2), neonatal asphyxia can lead to thrombocytopenia, disseminated intravascular coagulation and platelet dysfunction (3,4). The hemostatic derangement may increase the risk of intracranial hemorrhage, bleeding tendency and thromboembolism (2). Platelet activation may contribute to the platelet dysfunction and could be involved in the mechanism of hypoxic-ischemic encephalopathy leading to adverse neurodevelopmental outcome (5). Indeed, hypoxia alone and with reoxygenation can lead to platelet activation and dysfunction (3,6).

Upon activation, platelet aggregation is mediated by the release of thromboxane A_2, matrix metalloproteinases (MMPs) and adenosine diphosphate (3,7,8). We recently reported that hypoxia followed by reoxygenation with 100% oxygen was associated with platelet aggregatory dysfunction and an increase in the plasma thromboxane B_2 (TxB_2) concentration (6), which is a stable metabolite of thromboxane A_2. Matrix metalloproteinases are zinc-dependent endopeptidases that play an important role in the regulation of platelet aggregation (9). While MMP-2 (constitutive isoform, 72 kDa) stimulates platelet aggregation (7), MMP-9 (inducible isoform, 92 kDa) inhibits platelet aggregation (8). Platelet dysfunction after hypoxia-reoxygenation (H-R) injury could be related to oxidative stress and the generation of free radicals (superoxide anion and hydroxyl radical) (10). Furthermore, reactive nitrogen species such as peroxynitrite anion can cause platelet activation and aggregatory dysfunction (11) and known to activate MMPs (12). Cyclic adenosine 3',5' monophosphate (cAMP) and cyclic 3',5' monophosphate (cGMP) are secondary messengers that regulate platelet function (13).

Up-regulation of intracellular cAMP and cGMP is involved in the inhibition of platelet function (14).

Dobutamine is a selective β-adrenoceptor agonist (15,16). It is commonly used to treat hypotension and myocardial dysfunction at a dose of 5-20 μg/kg/min in asphyxiated neonates (17). It is known that platelets have β-adrenoceptors on their cell membrane (18,19). Therefore, dobutamine may interact with the β-adrenoceptors on the surface of platelets and alter the platelet function. In adults undergoing dobutamine stress echocardiography, Lewis et al noticed an increased risk of sustaining non-fatal myocardial infarction (20). They demonstrated that this could be related to the platelet activation secondary to dobutamine infusion during the test (21). However, there is no data in the literature about the effect of dobutamine on platelet function when the drug is being given to asphyxiated neonates. Therefore, we tested the hypothesis that the administration of dobutamine could aggravate the platelet activation and aggregatory dysfunction during reoxygenation after hypoxia. We also examined the plasma levels of markers of the related mechanistic pathways that regulate the platelet aggregatory function including TxB_2 (prostaglandin), cAMP, cGMP (nitric oxide-guanylyl cyclase), nitrotyrosine (nitric oxide-peroxynitrite), MMP-2 and MMP-9.

Methods

The experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Health Science Animal Policy and Welfare Committee, University of Alberta.

Animal preparation

Thirty-eight mixed-breed newborn piglets (age: 1-3 day, weight: 1.5-2.3 kg) were brought in from a local farm on the same day of experimentation. Piglets were anesthetized initially with halothane while spontaneously breathing and then given i.v. fentanyl (10 μg/kg/h), midazolam (0.1-0.2 mg/kg/h), and pancuronium (0.05-0.1 mg/kg/h) once the i.v. access was secured and mechanical ventilation through tracheostomy initiated. Additional boluses of fentanyl (10 μg/kg) and pancuronium (0.05 mg/kg) were given as needed. A dose of i.v. acepromazine (0.25 mg/kg) was also given. The animals were ventilated by a Sechrist infant ventilator (Model IV-100; Sechrist Industries, Anaheim, CA) set at pressures of 18/4 cm H_2O, rates of 15-20 breaths/min, and fractional inspired oxygen concentration (FiO_2) of 0.21-0.25. The aim was to maintain oxygen saturation of 90% to 100% and $PaCO_2$ of 35-45 mmHg. Oxygen saturation was continuously monitored with a pulse oximeter (Nellcor, Hayward, CA). Femoral venous and arterial central catheters (3.5 or 5 Fr; Sherwood Medical Co., St. Louis, MO) were inserted to give fluids and drugs, and to monitor mean systemic arterial pressure (SAP) and blood sampling, respectively. Heart rate and SAP were measured with a Hewlett Packard 78833B monitor (Hewlett Packard Co., Palo Alto, CA). Maintenance fluid consisted of 10% dextrose-saline at 10 ml/kg/h. The piglet's temperature was maintained at 38.5–39.5°C using a heating pad. Stabilization, a minimum of 30 min after surgery, was defined as the change in heart rate and SAP <10%, normal arterial acid-base balance and blood gas analysis (pH: 7.35-7.45; PO_2: 60-100 mmHg; $PaCO_2$: 35-45 mmHg).

Experimental protocol

After stabilization, normocapnic alveolar hypoxia (FiO_2=0.10-0.15) was induced in piglets for 2h aiming for arterial oxygen saturations of 30-40% and severe metabolic acidosis. This was followed by reoxygenation with 100% oxygen for 1h and then 21% oxygen for 3h. Piglets were block-randomized to receive dobutamine (Dobutrex, Eli Lilly Inc., Scarborough, ON, Canada) at a fixed dose (5, 10, or 20 μg/kg/min) or saline (control) starting at 2h of reoxygenation (for 2h of infusion) (n=8 in each group). Sham-operated animals (n=6) received saline and were not exposed to H-R. At the end of the study, piglets were euthanized with an i.v. injection of pentobarbital (100 mg/kg).

Platelet count and platelet function

Platelet number and function were studied at baseline and at the end of H-R (after 2h of dobutamine or saline infusion) and the corresponding timepoints in the sham-operated group. Two ml of whole blood was collected in 3.15% Na citrate (9:1, vol:vol). Platelet count was determined using an automated counter (MicroDiff 16, Coulter, Hialeah, FL). Platelet aggregatory function was measured by collagen-stimulated whole blood impedance aggregation using a Chronolog Aggregometer (Chronolog Corp., Havertown, PA). Collagen (2, 5 or 10 μg/10μL) was added to citrated whole blood (495 μL) and normal saline (495 μL). The test was performed according to the manufacturer recommendations within 30 min of blood sampling (6). Previous pilot studies with collagen up to 20 μg indicated a maximum aggregatory response could be elicited at 10 μg/ml of collagen in platelets during H-R. We defined E_{max} as the maximum aggregatory response to collagen of 2-10 μg/ml and EC_{50} as the concentration of collagen needed to cause 50% of maximum aggregatory response to 2, 5 or 10 μg/ml of collagen.

Blood collection

Arterial blood samples were also taken at normoxic baseline, 2h of hypoxia, 2h of reoxygenation and 2h of dobutamine infusion to determine blood gas analysis, plasma concentrations of TxB_2, cAMP, cGMP, nitrotyrosine and plasma MMP-2 and -9 activities. Total blood taken for all tests was ≤ 9 ml which was replaced with an equal volume of normal saline.

Determination of plasma protein concentration

Platelet poor plasma was prepared by centrifugation at 10,000g for 15 min and stored at -80°C for subsequent biochemical analysis. The plasma protein concentration was assessed using a bicinchoninic acid protein assay kit (Catalog No. BCA1, Sigma, St. Louis, MO).

Determination of plasma concentrations of TxB_2, cAMP, cGMP and nitrotyrosine

Thromboxane B_2 is a stable metabolite of thromboxane A_2 and the plasma levels of this eicosanoid were assayed using a commercially available immunoassay kit (Catalog No. DE0700, R&D Systems Inc., Minneapolis, MN). The assay of plasma concentrations of cAMP and cGMP were performed using commercially available immunoassay kits (Catalog No. 581001 and 581021, respectively, Caymon Chemical Company, Ann Arbor, MI.). Nitrotyrosine is the major product obtained by the nitration of tyrosine by peroxynitrite (22). Nitrotyrosine can be used to assess systemic injury related to peroxynitrite (23). The plasma concentration of nitrotyrosine was performed using ELISA kit (Catalog No. HK501, Hycult Biotechnology Bv, Uden, The Netherlands).

Determination of plasma MMP-2 and -9 activities

Matrix metalloproteinase-2 and -9 activities in plasma were studied by gelatin zymography as previously described with minor modifications (6). Briefly, the samples were mixed with 6x sample buffer and electrophoresed on 8% SDS-PAGE (0.75mm thickness) containing gelatin (2 mg/ml) using mini-PROTEAN 3 system (Bio-Rad, Hercules, CA). Each sample (contains 20 μg of plasma protein) was analyzed in duplicate. Human recombinant MMP-2 and -9 were used as standards. Following electrophoresis, the gels were washed three times (20 min each) in 2.5% Triton-X 100. The gels were incubated in the incubation buffer (0.05 M Tris-HCl [Invetrogen, Carlsbad, CA], pH 7.5 containing 0.01 M $CaCl_2$, 0.2 M NaCl [Merk, Darmstadt, Germany] and 0.05% NaN_3) at 37°C for 20h. The gels were stained with 0.05% Coomassie brilliant blue G-250 in 25% methanol and 10% acetic acid for 2h shaking at room temperature, and then destained with 4% methanol and 8% acetic acid for 30 min. Finally the gels were rinsed in double-distilled water prior to drying between sheets of cellophane. The zymograms were scanned using PowerLook 1000 scanner (UMAX, Dallas, TX) and the gelatinolytic bands were analyzed using Quantity One 1-D analysis software (Bio-Rad, Hercules, CA).

Statistical analysis

Results are expressed as mean±SD or median [IQR] for parametric and nonparametric variables, respectively. Changes in the plasma concentrations of TxB_2, cAMP, cGMP, nitrotyrosine and plasma activities of MMP-2 and -9 and were expressed as the percentage of respective baseline values. The platelet count and aggregatory function and plasma markers were analyzed using paired *t*-test to compare the difference between respective values at baseline and the end of H-R. Wilcoxon signed rank rest was used for non-parametric variables. One way analysis of variance was also used to compare differences of means between groups at each time point with Fisher's least significant difference test for *post hoc* analysis. Kruskal-Wallis test with Dunn's test for *post hoc* analysis were used to compare the differences between groups for nonparametric variables. A statistic software (SigmaStat, v.2.0, Jandel Corp., San Rafael, CA) was used. Significance was defined as $p< 0.05$.

Results

There were no significant differences between groups in age, weight and sex distributions (data not shown).

At baseline, all groups had similar heart rate, SAP and PaO_2 and normal acid-base balance (Tables 7-1 and 7-2). Two hours of hypoxia (mean PaO_2: 31-36 vs. 74±11 mmHg in shams; $p<0.001$, ANOVA) resulted in a severe metabolic acidosis (mean pH: 7.02-7.06 vs. 7.37±0.06 in shams; $p<0.001$) and hypotension (mean SAP: 27-36 vs. 66±10 mmHg in shams; $p<0.001$) (Tables 7-1 and 7-2). There was no significant change on heart rate at the end of hypoxia. At 2h of reoxygenation, the SAP recovered partially (mean SAP: 40-48 mmHg) and pH normalized. After 2h of dobutamine or saline infusion, the heart rate, SAP and acid-base balance were not different from those of controls.

Platelet count and function

At 4h of reoxygenation, platelet counts of all H-R groups decreased significantly (213±87 vs. 323±90 x 10^9/L of baseline). Compared to respective baselines, platelet counts decreased significantly in control, 10 and 20 μg/kg/min dobutamine-treated groups ($p<0.05$, paired *t*-test) but modestly in sham and 5 μg/kg/min dobutamine-treated groups ($p<0.1$) (Figure 7-1). At the end of the experiment, 6 piglets had platelet count <150 x 10^9/L (one animal in sham, 5, 10 and 20 μg/kg/min dobutamine and two animals in control group). There was no animal with platelet count below 100 x 10^9/L at anytime. There were no significant differences in platelet count between all groups at baseline or at 4h of reoxygenation after hypoxia (2h of dobutamine infusion).

After 2h of dobutamine infusion (4h of reoxygenation), the whole blood collagen-induced platelet aggregation in piglets receiving 10 and 20 μg/kg/min of dobutamine was impaired significantly from the respective baseline responses (Figures 7-2D and 7-2E). The aggregation curve was shifted to the right which was characterized by higher EC_{50} but no significant change in the E_{max} (Table 7-3). Compared to respective baselines, the EC_{50} increased to 5.4±1.6 and 4.6±2.0 μg/ml in piglets receiving 10 and 20 μg/kg/min of dobutamine (vs. 2.4±1.4 and 2.5±0.8 μg/ml, of the respective baseline, $p<0.05$, paired *t*-test) (Table 7-3). Apart from a modest increase in the EC_{50} of the control group ($p=0.1$, paired *t*-test) (Table 7-3, 7-2B), there were no significant changes in platelet aggregation in shams or 5 μg/kg/min dobutamine-treated group at the end of H-R compared to respective baselines (Table 7-3, Figures 7-2A and 7-2C). There were no significant differences in the EC_{50} or E_{max} between groups at baseline or at the end of H-R.

Plasma concentrations of TxB_2, cAMP, cGMP and nitrotyrosine and plasma activities of MMP-2 and -9

At 2h of dobutamine infusion, the plasma TxB_2 concentration of 20 μg/kg/min dobutamine-treated group was significantly increased from baseline (Table 7-4) ($p<0.05$, Wilcoxon test). There was a trend toward higher plasma TxB_2 concentrations in the 10 and 20 μg/kg/min dobutamine-treated groups ($p=0.09$, Kruskal-Wallis test). There were no significant differences between all groups and within group in the plasma concentrations of cAMP and cGMP.

There was a significant decrease in the plasma concentration of nitrotyrosine in shams and 10 μg/kg/min dobutamine treated group at 2h of dobutamine infusion ($p<0.05$, Wilcoxon test) (Table 7-4). However, there were no significant differences between groups at baseline or at 2h of dobutamine infusion.

At 2h of hypoxia, plasma MMP-9 activities were significantly higher (median: 113-177% of baseline) in the hypoxic piglets than that of shams (median: 56 [20-66] % of baseline; $p<0.05$, Kruskal-Wallis test) (Figure 7-3A). The plasma MMP-9 activity then fell during the reoxygenation phase with no difference between groups and normalized to the baseline values at the end of H-R or 2h of dobutamine infusion. There were no significant changes or differences in the plasma activity of MMP-2 in the sham and H-R piglets during the experimental period (Figure 7-3B).

Discussion

In this study, we demonstrated a tendency for an impaired platelet aggregatory function *ex vivo* secondary to dobutamine infusion at moderate-high doses in a model of H-R in newborn piglets. This impairment was seen in the piglets receiving 10 and 20 but not 5 μg/kg/min of dobutamine. The resultant rightward shifting of the collagen-induced response curve was associated with increased EC_{50} and unchanged E_{max}. We were not able to demonstrate any significant changes in the plasma concentrations of cAMP, cGMP and nitrotyrosine, and plasma MMP-2 and -9 activities secondary to dobutamine infusion. However, we noticed a trend of increased plasma TxB_2 concentration during dobutamine infusion with a significant increase in the group receiving 20 μg/kg/min. These findings support a dose-dependent effect of dobutamine on platelet activation and aggregatory dysfunction during H-R in neonatal piglets.

There is limited data in the adult literature examining the interaction of dobutamine and platelets. Lewis el al reported that patients undergoing dobutamine stress echocardiography had a higher risk of sustaining non-fatal myocardial infarction than other patients undergoing treadmill stress test (20). Galloway et al then found that dobutamine infusion given during dobutamine stress echocardiography test may stimulate platelets and leads to activation (21). The data of our study support such an interaction on the neonatal platelets and demonstrate platelet activation with increased TxB_2 concentrations during high dose dobutamine infusion in H-R.

Asphyxia, hypoxia and H-R can lead to platelet activation and dysfunction (3,4,6,24). Similar effect of H-R on the platelet aggregatory function was observed in our study. The infusion of dobutamine may however exacerbate the effect of H-R leading to significant platelet aggregatory dysfunction as seen in 10 and 20 μg/kg/min of dobutamine. The significant rightward shifting of the dose-response curve may suggest decreased sensitivity towards the collagen stimulation. The pharmacodynamic and/or pharmacokinetic changes may occur at the agonist-receptor level and/or signaling pathway. The resultant platelet activation (increased TxB_2) with dobutamine infusion can render the platelets insensitivity to further collagen stimulation *ex vivo*.

Recently, we have shown the use 100% oxygen in newborn piglets with H-R was associated with platelet aggregatory dysfunction and increased plasma MMP-9 but not MMP-2 activities (6). Similarly in the current study, we observed a higher plasma MMP-

9 activities but not MMP-2. However, the significant increase in the plasma MMP-9 activities was observed only at the end of 2h of hypoxia. Fernandez-Patron et al found that platelet aggregation can be inhibited by MMP-9 (8). The above evidence may also suggest that platelet aggregatory dysfunction in our model might be partly due to excessive release of MMP-9.

Other mechanisms including oxidative stress may also activate platelets (10). Oxygen superoxide reacts rapidly with nitric oxide to form peroxynitrite which also can cause platelet activation and dysfunction (11). Nitrotyrosine is used to assess systemic tissue injury related to peroxynitrite-induced oxidative stress. In the sham-operated animals, we observed a significant decrease in the plasma nitrotyrosine concentrations suggesting resolution of the inflammatory condition following surgical instrumentation. However, we did not find any significant difference in the plasma concentration of nitrotyrosine between groups. This suggests dobutamine infusion may not increase the peroxynitrite oxidative stress.

We also found a lower platelet count after H-R (2h after dobutamine infusion). This could be at least in part due to the surgery and/or the H-R protocol (6). There were significant decrease in platelet counts in control, 10 and 20 μg/kg/min dobutamine-treated groups (6,25,26). Interestingly, Galloway et al found a slight but significant decrease in platelet count during dobutamine stress echocardiography (21).

Various drugs stimulate or inhibit platelet function by via the cAMP (e.g. prostaglandin) and cGMP (e.g. nitric oxide) pathways (13). Both cAMP and cGMP are metabolized by the enzyme phosphodiesterase. High intracellular levels of cAMP and cGMP inhibit platelet function. In our experiment, dobutamine might lead to platelet inhibition by stimulating the β-receptors on the platelet cell membrane (19) and lead to activation of adenylyl cyclase which in turn up-regulate the intracellular cAMP concentration. Due to the technical difficulty to measure intra-platelet nucleotides, we measured the plasma concentration of cAMP instead and did not observe any significant differences between groups. This is because the plasma cAMP concentration does not necessarily reflect the intracellular levels in the platelet. Measuring the intracellular concentration of this nucleotide in the future might give a better understanding of the mechanistic pathway of platelet inhibition during dobutamine infusion.

Clinical implications

We are not certain about the implication of impaired platelet aggregatory function, to collagen stimulation *ex vivo*. Although the platelet function in vivo may need further study, our results suggest impaired platelet function associated with platelet activation. Asphyxiated neonates may suffer from various complications including cardiovascular and hemostatic dysfunction. Inotropes, usually given for hours or days, are used to treat myocardial dysfunction and may be associated with adverse effects which will influence the choice. Dobutamine, β-adrenergic, may activate platelets and impair aggregatory function at moderate to high doses. The consequences of this platelet pathology in asphyxiated neonates need further investigation. Until then, dobutamine and other inotropes should be used with caution in the sick infant since they may affect the platelet aggregatory function.

Conclusions

The use of moderate to high doses of dobutamine to treat post-asphyxial hypotension activates the platelet and leads to platelet aggregatory dysfunction in newborn piglets with hypoxia and reoxygenation. Judicious use of inotropes in sick neonates is recommended.

Table 7-1: Heart rate and mean systemic arterial pressure (SAP) in control (saline) and dobutamine-treated (5, 10 and 20 μg/kg/min) groups at baseline, after 2h of hypoxia and 4h of reoxygenation (2h of dobutamine infusion) (n=8 each). The sham-operated group had no hypoxia and reoxygenation (n=6). Values are mean±SD.

	Sham	Control	Dobutamine (μg/kg/min)		
			5	10	20
Baseline					
Heart rate (beats/min)	184±39	214±32	202±39	226±45	201±38
SAP (mmHg)	69±17	73±13	74±15	73±7	74±14
2h Hypoxia					
Heart rate (beats/min)	193±41	193±12	215±32	209±16	217±23
SAP (mmHg)	66±10	28±3*	32±3*	27±4*	36±11*
2h Reoxygenation					
Heart rate (beats/min)	190±30	205±30	237±40	244±33*	210±27
SAP (mmHg)	54±7	40±8*	40±9*	40±9*	48±8
2h Dobutamine or saline infusion					
Heart rate (beats/min)	194±18	240±36*	251±34*	253±23*	236±34*
SAP (mmHg)	50±5	39±8	39±5	40±9	46±12

*p<0.05 vs. shams (ANOVA)

Table 7-2: Arterial blood pH, partial pressure of oxygen (PaO_2) and bicarbonate (HCO_3^-) in control (saline) and dobutamine-treated (5, 10 and 20 µg/kg/min) groups at baseline, after 2h of hypoxia and 4h of reoxygenation (2h of dobutamine infusion) (n=8 each). The sham-operated group had no hypoxia and reoxygenation (n=6). Values are mean±SD.

	Sham	Control	Dobutamine (µg/kg/min)		
			5	10	20
Baseline					
pH	7.37±0.04	7.43±0.04*	7.39±0.03	7.36±0.03	7.36±0.04
PaO_2 (mmHg)	67±10	71±14	65±6	70±10	70±8
HCO_3^- (mmol/L)	25±2	23±3	22±3	23±3	24±2
2h Hypoxia					
pH	7.37±0.06	7.05±0.09*	7.02±0.16*	7.03±0.07*	7.06±0.09*
PaO_2 (mmHg)	74±11	31±8*	33±6*	36±7*	31±8*
HCO_3^- (mmol/L)	24±3	11±3*	9±4*	9±3*	11±3*
2h Reoxygenation					
pH	7.38±0.05	7.35±0.05	7.34±0.05	7.32±0.04	7.34±0.08
PaO_2 (mmHg)	71±6	62±12	69±11	64±8	63±8
HCO_3^- (mmol/L)	23±2	21±2	23±3	20±2	21±3
2h Dobutamine or saline infusion					
pH	7.37±0.06	7.32±0.06	7.32±0.07	7.30±0.05	7.34±0.08
PaO_2 (mmHg)	64±6	61±9	66±12	63±9	59±4
HCO_3^- (mmol/L)	23±2	20±3	20±3	18±3	21±3

*$p<0.05$ vs. shams (ANOVA)

Table 7-3: Collagen-stimulated whole blood aggregatory responses in control (saline) and dobutamine-treated (5, 10 and 20 μg/kg/min) groups at baseline and 4h of reoxygenation (2h of dobutamine infusion) (n=8 each). The sham-operated group had no hypoxia and reoxygenation (n=5). Values are mean±SD.

	Sham	Control	Dobutamine (μg/kg/min)		
			5	10	20
EC_{50} (μg/ml)					
Baseline	2.1±1.	2.8±1.7	3.0±1.7	2.4±1.4	2.5±0.8
End of H-R	3.9±1.9	4.5±2.0†	3.8±1.8	5.4±1.6*	4.6±2.0*
E_{max}(Ω)					
Baseline	37±9	37±6	36± 13	31± 9	32± 6
End of H-R	40±14	31±10	37± 14	35±10	27± 10

EC_{50}: concentration of collagen needed to cause 50% of maximum aggregatory response to collagen concentration of 2, 5 or 10 μg/ml.

E_{max}: Maximum aggregatory response to 2-10 μg/ml of collagen.

End of H-R: End of hypoxia-reoxygenation (2h of dobutamine or saline infusion).

* p<0.05 and † p=0.1 vs. baseline; paired *t*-test.

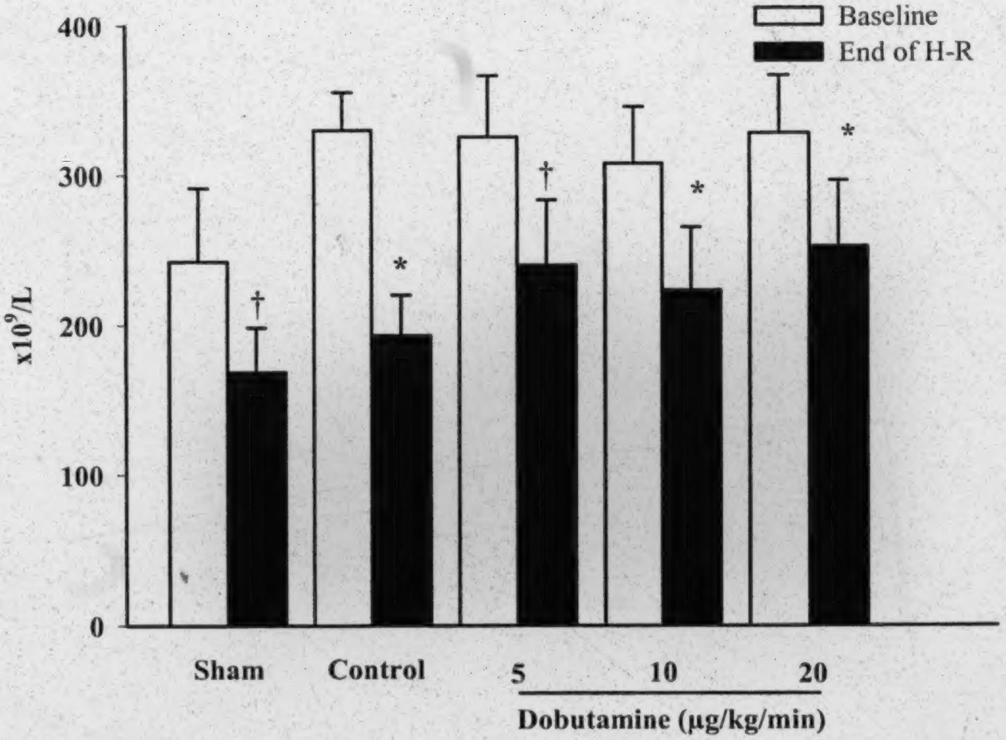
Table 7-4: Plasma concentrations of thromboxane B_2 (TxB_2), cAMP, cGMP and nitrotyrosine in control (saline) and dobutamine-treated (5, 10 and 20 µg/kg/min) groups at 4h of reoxygenation (2h of dobutamine infusion or saline infusion) (n=8 each). The sham-operated group had no hypoxia and reoxygenation. Data are expressed per percentage of baseline (median [IQR]).

	Sham	Control	Dobutamine (µg/kg/min)		
			5	10	20
TxB_2	97 [39-359]	87 [57-102]	59 [50-129]	164 [68-337]	201 [110-491]* †
cAMP	299 [142-320]	133 [65-243]	162 [55-246]	224 [52-355]	123 [56-1141]
cGMP	114 [102-160]	70 [57-86]	94 [90-111]	115 [96-163]	84 [64-108]
Nitrotyrosine	76 [55-84]*	76 [46-126]	91 [81-133]	76 [67-98]*	118 [95-125]

*$p<0.05$ vs. baseline (paired *t*-test or Wilcoxon test)

†$p<0.1$ (Kruskal-Wallis test)

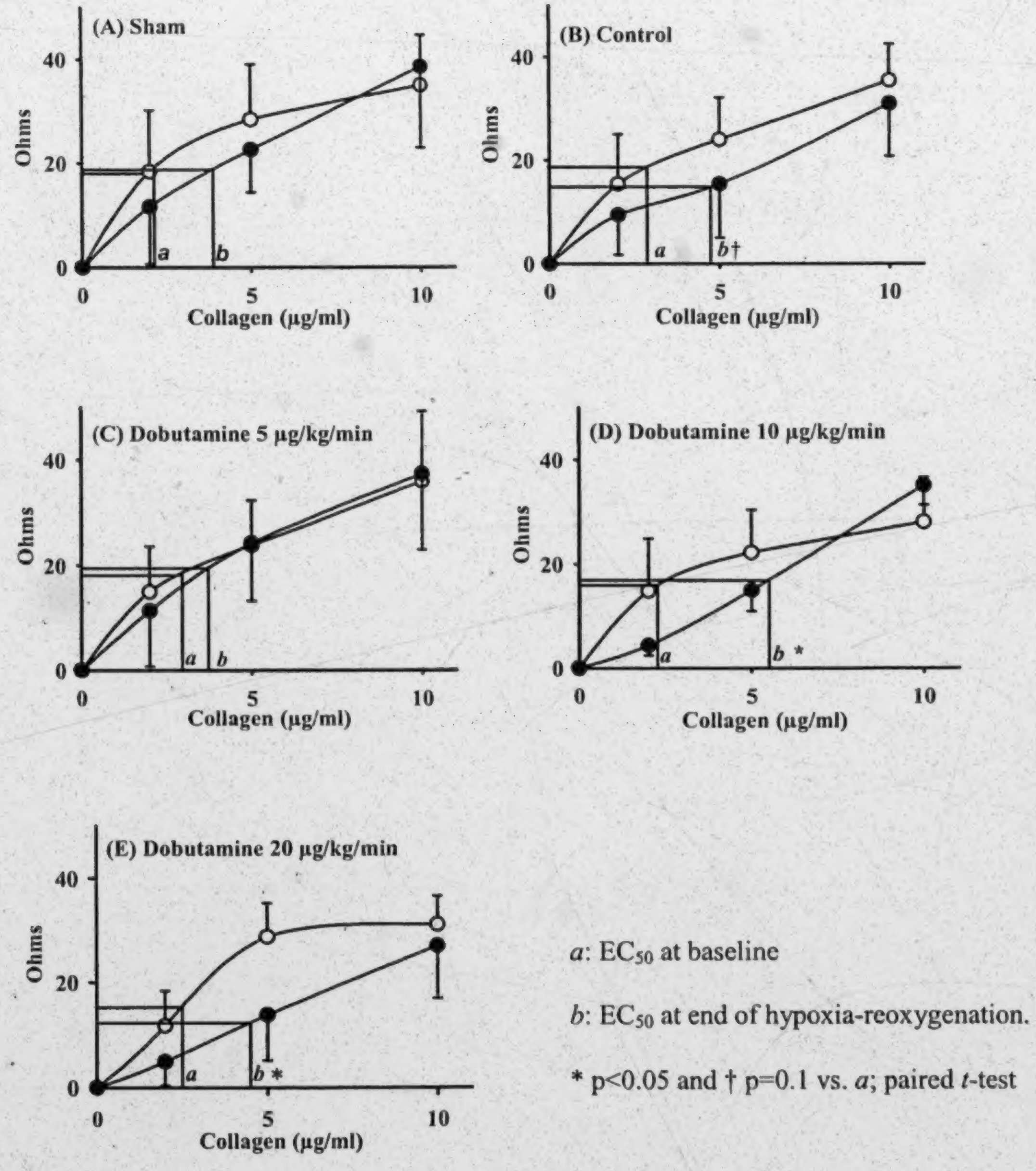
Figure 7-1: Platelet counts in control (saline) and dobutamine-treated (5, 10 and 20 μg/kg/min) groups at baseline and at the end of hypoxia and reoxygenation (H-R) (2h of dobutamine or saline infusion) (*n*=8 each). The sham-operated group had no H-R (n=6). Error bars represent standard deviation.



*p<0.05 vs. baseline; paired *t*-test

† p<0.1 vs. baseline; paired *t*-test.

Figure 7-2: Collagen-stimulated whole blood aggregatory responses in (A) sham, (B) control and (C) 5, (D) 10 and (E) 20 μg/kg/min of dobutamine at baseline (○) and at the end of hypoxia-reoxygenation (●) (2h of dobutamine or saline infusion). Error bars represent standard deviation.

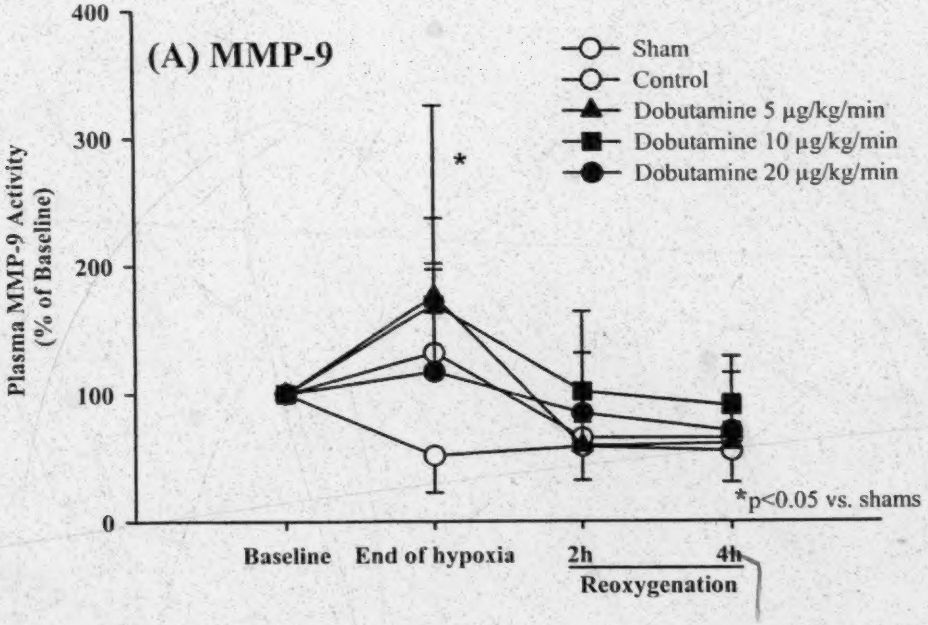


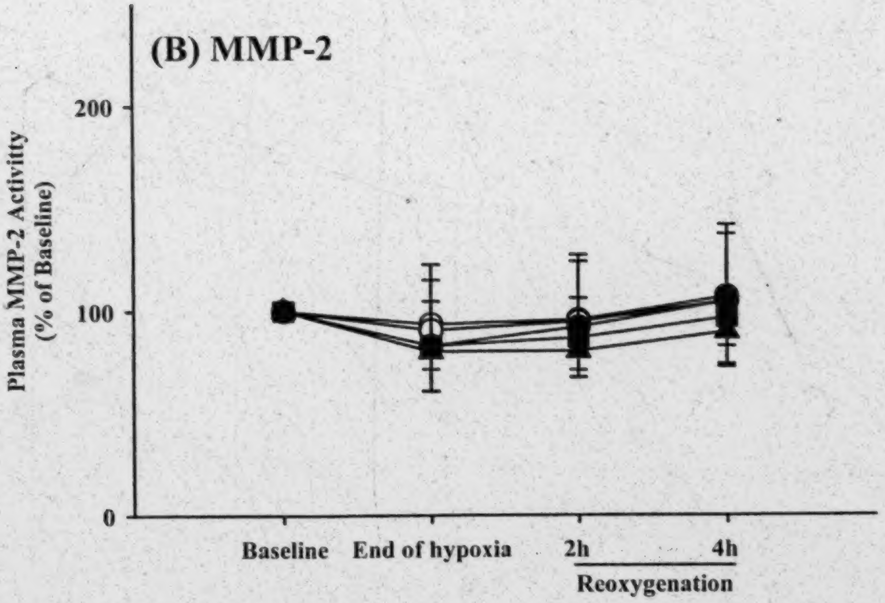
a: EC_{50} at baseline

b: EC_{50} at end of hypoxia-reoxygenation.

* $p<0.05$ and † $p=0.1$ vs. *a*; paired *t*-test

Figure 7-3: Temporal changes in the plasma matrix metalloproteinase (MMP)-9 (A) and MMP-2 (B) activities. Error bars represent standard deviation.





References

1. Black RE, Morris SS, Bryce J 2003 Where and why are 10 million children dying every year? Lancet 361:2226-2234
2. Martin-Ancel A, Garcia-Alix A, Gaya F, Cabanas F, Burgueros M, Quero J 1995 Multiple organ involvement in perinatal asphyxia. J Pediatr 127:786-793
3. Suzuki S, Morishita S 1998 Hypercoagulability and DIC in high-risk infants. Semin Thromb Hemost 24:463-466
4. Fichera A, Pratico G, Sciacca F 1989 Neonatal hypoxia and hemocoagulative changes. Pediatr Med Chir 11:643-647
5. Akisu M, Kultursay N, Coker I, Huseyinov A 1998 Platelet-activating factor is an important mediator in hypoxic ischemic brain injury in the newborn rat. Flunarizine and Ginkgo biloba extract reduce PAF concentration in the brain. Biol Neonate 74:439-444
6. Cheung PY, Stevens JP, Haase E, Stang L, Bigam DL, Etches W, Radomski MW 2006 Platelet dysfunction in asphyxiated newborn piglets resuscitated with 21% and 100% oxygen. Pediatr Res 59:636-640
7. Sawicki G, Salas E, Murat J, Miszta-Lane H, Radomski MW 1997 Release of gelatinase A during platelet activation mediates aggregation. Nature 386:616-619
8. Fernandez-Patron C, Martinez-Cuesta MA, Salas E, Sawicki G, Wozniak M, Radomski MW, Davidge ST 1999 Differential regulation of platelet aggregation by matrix metalloproteinases-9 and -2. Thromb Haemost 82:1730-1735
9. Hooper NM 1994 Families of zinc metalloproteases. FEBS Lett 354:1-6

10. Leo R, Pratico D, Iuliano L, Pulcinelli FM, Ghiselli A, Pignatelli P, Colavita AR, FitzGerald GA, Violi F 1997 Platelet activation by superoxide anion and hydroxyl radicals intrinsically generated by platelets that had undergone anoxia and then reoxygenated. Circulation 95:885-891

11. Moro MA, rley-Usmar VM, Goodwin DA, Read NG, Zamora-Pino R, Feelisch M, Radomski MW, Moncada S 1994 Paradoxical fate and biological action of peroxynitrite on human platelets. Proc Natl Acad Sci U S A 91:6702-6706

12. Okamoto T, Akaike T, Nagano T, Miyajima S, Suga M, Ando M, Ichimori K, Maeda H 1997 Activation of human neutrophil procollagenase by nitrogen dioxide and peroxynitrite: a novel mechanism for procollagenase activation involving nitric oxide. Arch Biochem Biophys 342:261-274

13. Schwarz UR, Walter U, Eigenthaler M 2001 Taming platelets with cyclic nucleotides. Biochem Pharmacol 62:1153-1161

14. Haslam RJ, Dickinson NT, Jang EK 1999 Cyclic nucleotides and phosphodiesterases in platelets. Thromb Haemost 82:412-423

15. Ruffolo RR, Jr., Messick K 1985 Effects of dopamine, (+/-)-dobutamine and the (+)- and (-)-enantiomers of dobutamine on cardiac function in pithed rats. J Pharmacol Exp Ther 235:558-565

16. Ruffolo RR, Jr. 1987 The pharmacology of dobutamine. Am J Med Sci 294:244-248

17. Noori S, Friedlich P, Seri I 2004 Pharmacology Review: The use of dobutamine in the treatment of neonatal cardiovascular compromise. NeoReviews 5:e22-e26

18. Ganchev TS, Negrev NN 1989 Beta1- and beta2-adrenergic effects on thrombocytopoiesis in rats. Gematol Transfuziol 34:50-53

19. Ganchev T, Negrev N, Ilkova R 1990 Adrenergic dependence of the platelet aggregation in rats. Acta Physiol Pharmacol Bulg 16:46-49

20. Lewis WR, Arena FJ, Galloway MT, Bommer WJ 1997 Acute myocardial infarction associated with dobutamine stress echocardiography. J Am Soc Echocardiogr 10:576-578

21. Galloway MT, Paglieroni TG, Wun T, Arena FJ, Lewis WR 1998 Platelet activation during dobutamine stress echocardiography. Am Heart J 135:888-900

22. Halliwell B 1997 What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo? FEBS Lett 411:157-160

23. Alvarez B, Radi R 2003 Peroxynitrite reactivity with amino acids and proteins. Amino Acids 25:295-311

24. Akisu M, Kultursay N, Coker I, Huseyinov A 1998 Plasma platelet-activating factor levels in newborn infants with and without perinatal asphyxia: Is it an additional marker of perinatal asphyxia? Acta Paediatr Jpn 40:427-431

25. Castle V, Coates G, Mitchell LG, O'Brodovich H, Andrew M 1988 The effect of hypoxia on platelet survival and site of sequestration in the newborn rabbit. Thromb Haemost 59:45-48

26. Roberts I, Murray NA 2003 Neonatal thrombocytopenia: causes and management. Arch Dis Child Fetal Neonatal Ed 88:F359-F364

CHAPTER 8

The myocardial matrix metalloproteinase-2 and -9 and nitrotyrosine levels in newborn piglets with hypoxia and reoxygenation during dobutamine infusion

Abstract

Background: During reoxygenation after hypoxia, peroxynitrite is produced and MMP-2 is activated, which may contribute to the myocardial dysfunction. Little is known if these factors are affected when dobutamine is given to treat shock in asphyxiated neonates. We examined if dobutamine would increase these markers of oxidative stress in the myocardium, when it is given to newborn piglets to treat myocardial dysfunction secondary to hypoxia and reoxygenation.

Methods: Mechanically ventilated newborn piglets were exposed to hypoxia for 2h and resuscitated with 100% for 1h and then 21% oxygen for 3h. At 2h of reoxygenation, the piglets were given dobutamine infusion for 2h at fixed dose (0, 5, 10 or 20 μg/kg/min). Sham-operated animals were included in the study. Hemodynamic variables, blood gases and acid base-balance were determined at specific timepoints. The left ventricular (LV) matrix metalloproteinase (MMP)-2 and -9 activities were determined by gelatin zymography whereas nitrotyrosine concentration was determined by ELISA.

Results: Hypoxia caused severe metabolic acidosis and shock. The acidosis recovered after 2h of reoxygenation and remained normal thereafter. The cardiac index and systemic arterial pressure recovered partially upon reoxygenation but continued to decline prior to dobutamine infusion. Dobutamine improved the cardiac index significantly in piglets receiving 20 μg/kg/min dobutamine but had no effect on systemic arterial pressure. Dobutamine infusion did not have any effect on the gelatinolytic activities of MMP-2 and -9 nor nitrotyrosine concentration of the LV.

Conclusions: In a neonatal swine model of hypoxia and reoxygenation, dobutamine infusion for a short period was not associated with increased MMP-2 and -9 and nitrotyrosine levels in the myocardium.

7

Introduction

Myocardial dysfunction can be found in approximately one third of asphyxiated neonates (1). During the process of resuscitation (reperfusion and reoxygenation), the neonate is exposed to oxidative stress (2) causing injuries such as myocardial dysfunction (3). Peroxynitrite is a reactive oxidant that is produced from the reaction of nitric oxide with superoxide anion. Peroxynitrite can impair the myocardial function through different mechanisms including a direct effect on the myocardium (4,5) or mediated by the activation of matrix metalloproteinases (MMPs) (6). Matrix metalloproteinases belong to the family of zinc-containing endopeptidases that are involved in the remodeling of the extracellular matrix during various physiological and pathological conditions (7,8). Matrix metalloproteinase-2 can lead to myocardial dysfunction by degrading troponin I (9) and myosin light chain (10). Matrix metalloproteinase-9 also contributes to the myocardial dysfunction and remodeling (11). Evaluation of these two MMPs could provide information on the oxidative stress-related injuries in the myocardium in asphyxiated neonates (12). Due to the short biological half life of peroxynitrite, its production is difficult to be measured. Therefore, the production of peroxynitrite in intact biological system is usually assessed by measuring nitrotyrosine (13), a product of protein nitration by peroxynitrite (14).

Asphyxiated neonates with myocardial dysfunction are often treated with inotropes to improve the cardiac function and enhance systemic oxygen delivery to the tissue (15). It is important for such inotropes not only to achieve these goals but also not to cause further damage to the body organs. In chapter 6, we showed that dobutamine improved the cardiac output and systemic oxygen delivery in a neonatal swine model of hypoxia and reoxygenation (H-R). In regard to oxidative stress-related injury, Miura et al found that adrenergic catechol derivatives of dobutamine inhibited lipid peroxidation (16). More recently, White et al found that short-term infusion of dobutamine improved functional status and reduced indices of oxidative stress including plasma nitrotyrosine in adult patients with decompensated congestive heart failure (17). To our knowledge, there are no data in the literature on the relationship between MMPs and nitrotyrosine and dobutamine infusion during neonatal asphyxia. Therefore, we examined this relationship. We measured the myocardial MMP-2 and-9 and nitrotyrosine levels in these H-R piglets.

Methods

The experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Health Science Animal Policy and Welfare Committee, University of Alberta.

Detailed methodology of the experiment is described in chapter 6. Briefly, newborn piglets (1-3 days, 1.4-2.3 kg) were anesthetized and acutely instrumented for continuous hemodynamic monitoring. An infra-renal aortic catheter and a pulmonary arterial Transonic® flow probe were positioned for continuous monitoring of the systemic arterial pressure (SAP) and pulmonary flow (surrogate of cardiac output), respectively. Cardiac index (CI) was expressed as ml/kg/min and calculated as pulmonary flow (ml/min) ÷ weight (kg). After stabilization they were exposed to hypoxia (10-15% oxygen) for 2h followed by reoxygenation with 100% oxygen for 1h then 21% for 3h. Piglets were block-randomized to receive dobutamine infusion (5, 10 or 20 μg/kg/min) or saline (control) at 2-4h of reoxygenation (n=8 each). The sham-operated group (n=6) received saline and was not exposed to H-R. At the end of the study, piglets were euthanized with an i.v. injection of pentobarbital (100 mg/kg).

Preparation of tissue homogenates

Immediately after euthanizing the animals, the heart was excised and a portion the left ventricle (LV) wall was snap-frozen in liquid nitrogen and stored at -80°C until biochemical analysis. Tissue homogenates of LV were prepared according to the method described by Schulze et al (18) with minor modifications. Briefly, frozen tissue was ground in liquid nitrogen with a mortar and pestle, and then 75 mg±10% LV tissue was homogenized in 200 μL of cold homogenization buffer (5 mM Tris-HCl, 320 mM sucrose, 1 mM diththreitol, 10 μg/ml leupeptin, 10 μg/ml soybean inhibitor, 2 μg/ml aprotinin) using a tissue homogenizer (PowerGen 125, Fisher Scientific, Pittsburgh, PA). Tissue homogenates were centrifuged at 10,000 rpm for 10 min at 4°C and then the supernatant was aspirated and stored at -80°C.

Determination of protein concentration

The protein concentration in the supernatant of tissue homogenates was assessed using a bicinchoninic acid protein assay kit (Catalog No. BCA1, Sigma, St. Louis, MO).

Determination of tissue activity levels of MMP-2 and -9

The activity levels of MMP-2 and -9 in tissue homogenates were determined by gelatin zymographic analysis as described by Kleiner and Stetler-Stevenson (19) with minor modifications. Briefly, the samples were mixed with 6x sample buffer and electrophoresed on 8% sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) (0.75mm thickness) containing gelatin (2 mg/ml) using mini-PROTEAN 3 system (Bio-Rad, Hercules, CA). Each sample (contains 20 μg of tissue protein) was analyzed in duplicate. The human recombinant MMP-2 and -9 (Calbiochem, San Diego, CA.) were used as standards. Following electrophoresis, the gels were washed three times (20 min each) in 2.5% Triton-X 100. The gels were incubated in the incubation buffer (0.05 M Tris-HCl [Invetrogen, Carlsbad, CA], pH 7.5 containing 0.01 M $CaCl_2$, 0.2 M NaCl [Merk, Darmstadt, Germany] and 0.05% NaN_3) at 37°C for 20h. The gels were stained with 0.05% Coomassie brilliant blue G-250 in 25% methanol and 10% acetic acid for 2h shaking at room temperature, and then destained with 4% methanol and 8% acetic acid for 30 min. Finally the gels were rinsed in double-distilled water prior to drying between sheets of cellophane. The zymograms were scanned using PowerLook 1000 scanner (UMAX, Dallas, TX) and the gelatinolytic bands were analyzed using Quantity One 1-D analysis software (Bio-Rad, Hercules, CA). The activity of gelatinolytic bands of MMP-2 and -9 are expressed in arbitrary units/mg protein.

Myocardial nitrotyrosine

The LV nitrotyrosine was determined by ELISA kit (Catalog No. HK501, Hycult Biotechnology Bv, Uden, The Netherlands). Briefly, homogenized LV samples were incubated in nitrotyrosine microtiter plates at room temperature for 1h and then washed 3 times with washing buffer. After that, tracer was added and incubated for another hour. Diluted streptavidin-peroxidase conjugate was then added and incubated for 1h. After washing with washing buffer, tetramethylbenzidine was added and incubated for 30 min. A purple color developed and the reaction was stopped by adding stop solution. The absorbance spectrum of the samples was determined by spectrophotometry at 450 nm and compared to known concentrations of nitrotyrosine. The level of LV nitrotyrosine was expressed as pmol/mg protein.

Statistical analysis

Results are expressed as mean±SD and median [IQR] for parametric and non-parametric values, respectively. One-way analysis of variance was used to compare differences of means between groups with Fisher's least significant difference test for *post hoc* analysis. Kruskal-Wallis test with Dunn's test for *post hoc* analysis was used to compare the differences between groups for non-parametric variables. Pearson correlation analysis was used to assess the relationship between cardiac index, myocardial MMPs activity levels and myocardial nitrotyrosine concentrations. A statistic software (SigmaStat, v.2.0, Jandel Corp., San Rafael, CA) was used. Significance was defined as $p<0.05$.

Results

Details of the acid-base balance, blood gas analyses and hemodynamic variables are mentioned in Chapter-6. Briefly, at 2h hypoxia (PaO_2 32±7 mmHg), shock, hypotension and metabolic acidosis developed similarly in all the groups (cardiac index: 94±4 ml/kg/min; SAP: 31±1 mm Hg and pH: 7.04±0.11 vs. shams, $p<0.05$) (Table 8-1). Ten minutes after reoxygenation, cardiac index and SAP improved but gradually deteriorated over the following 2h prior to starting dobutamine. The pH and PaO_2 also normalized in all groups. Cardiac index improved with 20 μg/kg/min of dobutamine (at 30 min of infusion: 188±37 vs. 131±31 ml/kg/min of controls, $p<0.05$; at 120 min of infusion: 172±35 vs. 119±33 ml/kg/min) and modestly in the 5 and 10 μg/kg/min treatment groups. The heart rate did not change. Dobutamine infusion had no effect on SAP.

Left ventricular myocardial MMP-2 and -9 activity levels

The gelatinolytic activity of MMP-2 and -9 was detected at 72 kDa and 92 kDa, respectively. After 2h of dobutamine infusion at 5-20 μg/kg/min, at 4h of reoxygenation following hypoxia, myocardial MMP-2, -9 and total MMPs activity levels were not different from those of controls and shams (Figures 8-2A and 8-2B). There were no significant associations between myocardial MMP-2, -9 or total MMPs activity levels and cardiac index.

Left ventricular nitrotyrosine levels

The LV nitrotyrosine level was not detectable in 2 sham-operated animals, 4 controls, 3, 3 and 4 animals in 5, 10 and 20 μg/kg/min dobutamine-treated groups, respectively. One animal from control had a very high LV nitrotyrosine concentration (84.8 pmol/mg protein) while the highest value in 5, 10 and 20 μg/kg/min dobutamine-treated groups were 17.3, 35.9 and 8.5 pmol/mg protein, respectively. There was no sham animal with a LV nitrotyrosine level above 4 pmol/mg protein. There were no significant differences in the LV nitrotyrosine level between studied groups (Figure 8-3). There was no significant association between LV nitrotyrosine levels and cardiac index.

Discussion

Our findings suggest that dobutamine infusion given to treat myocardial dysfunction in a neonatal swine model of H-R was not associated with further damage to the myocardium. This is supported by not finding any significant difference in the LV myocardial MMP-2 and -9 activities nor nitrotyrosine concentration between dobutamine-treated groups and those of controls.

Following hypoxia there is an immediate surge of oxygen free radicals generated upon reoxygenation (20-22). It is generally accepted that superoxide anions and hydroxyl free radicals are formed during the first seconds to minutes of reperfusion/reoxygenation and are involved in the subsequent development of poor myocardial contractility (23,24). Myocardial dysfunction in asphyxiated neonates might also be related to the MMPs activation by oxidative stress (6). Borke et al demonstrated, in a neonatal swine model of H-R, higher myocardial MMP-2 gelatinolytic activities in piglets resuscitated with 100% oxygen (12). Similarly, Haase et al found significant increases in the myocardial MMP-2 activities in piglets resuscitated 100% oxygen but no difference found in the myocardial MMP-9 activities (3). However, due to the different experimental design, we did not detect any significant difference in myocardial MMP-2 and -9 activity levels between H-R and sham-operated groups. The small sample size precludes us to study the effect of H-R or dobutamine infusion on the MMPs activities. Our study was primarily designed and powered to detect significant changes in hemodynamic variables. A sample size of 23 animals per group would be required in each group based on type 1 error of 0.05, power of 0.8 and the amount of variance in the MMPs activities.

Superoxide anions react with nitric oxide to form peroxynitrite anions at a diffusion limited rate (k=6.7 x 10^9 per mol/s), which is > 3 times faster than the enzymatic dismutation of superoxide anions catalyzed by superoxide dismutase (k=2 x 10^9 per mol/s) (25). Peroxynitrite anion is stable in alkaline solution (pH > 8.0) but injures cells and tissues when it is decomposed to highly reactive oxidant species following its rapid protonation at physiological pH (26). The current understanding on H-R injury, at least in part, lies on the generation of peroxynitrite during reoxygenation following hypoxia (27). Various researchers have demonstrated the detrimental effect of peroxynitrite and its biological roles which may include: lipid peroxidation (28), inhibition of mitochondrial respiration (29), stimulation of mitochondrial calcium efflux (30), oxidation of sulfhydryl groups (31), inhibition of aconitase activity (32), platelet aggregation and acute impairment of endothelium-dependent vasodilatation in coronary (33), systemic vasculature (34) and finally nitration of tyrosine resulting in nitrotyrosine (14). Nitrotyrosine has been used to assess peroxynitrite related injuries in both human diseases and animal models (14). In the neonatal population, the nitrotyrosine was used to explore the role of free radicals in the pathogenesis of periventricular leukomalacia (35) and bronchopulmonary dysplasia (13). We were not able to see any significant effect of H-R on the myocardial nitrotyrosine level. Apart from the small sample size and the wide variances, it is interesting that tissue nitrotyrosine is below detectable level in 16 of 38 (42%) of the LV samples. Due to inadequate LV tissue collection, these results could not be repeated. Nonetheless, if the results are genuine, our findings may suggest a very low signal of nitrosylated products at 4h of reoxygenation in the LV of newborn piglets.

Conclusions

In a neonatal swine model of H-R, the use of dobutamine to treat myocardial dysfunction was not associated with changes in the myocardial MMP-2, -9 and nitrotyrosine levels. Due to sample size and variance, interpretation of the results should be approached with caution. Further studies with larger sample size are warranted before making any recommendation.

Figure 8-1: Left ventricular myocardial matrix metalloproteinase (MMP) -2 activity in control (saline) and dobutamine-treated (5, 10 and 20 µg/kg/min) groups after 2h of hypoxia and 4h of reoxygenation (n=8 each). The sham-operated group had no hypoxia and reoxygenation (n=6). Median, 25th, 75th quartile range, 95% confidence interval and outliers are shown.

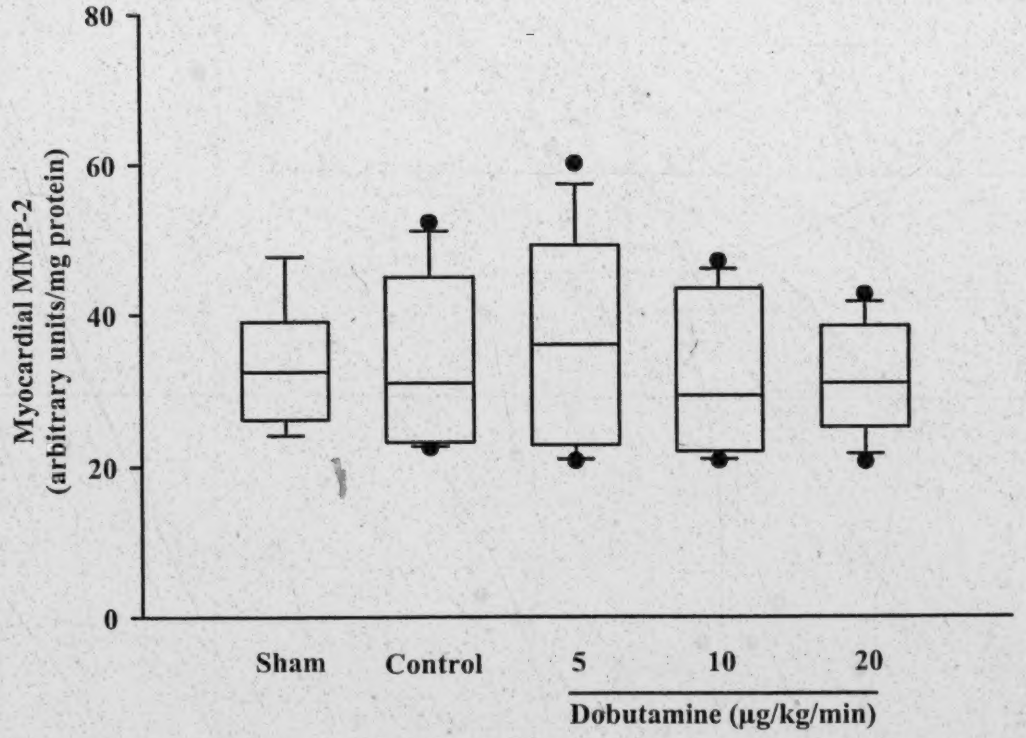


Figure 8-2: Left ventricular myocardial matrix metalloproteinase (MMP) -9 activity in control (saline) and dobutamine-treated (5, 10 and 20 µg/kg/min) groups after 2h of hypoxia and 4h of reoxygenation (n=8 each). The sham-operated group had no hypoxia and reoxygenation (n=6). Median, 25th, 75th quartile, 95% confidence interval and outliers are shown.

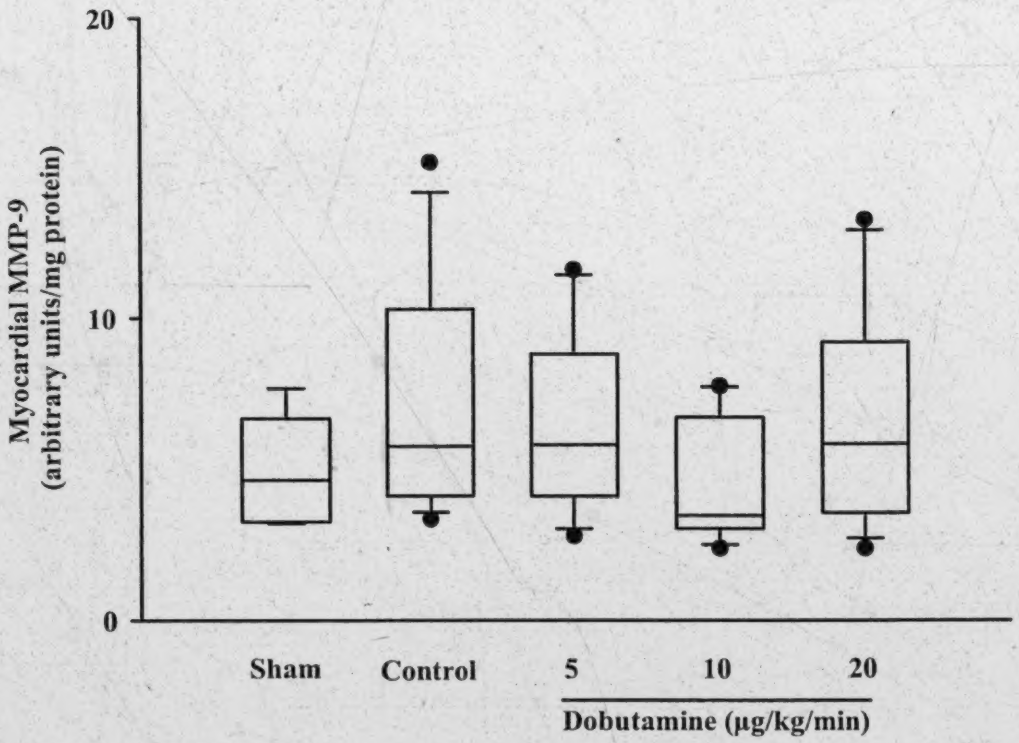
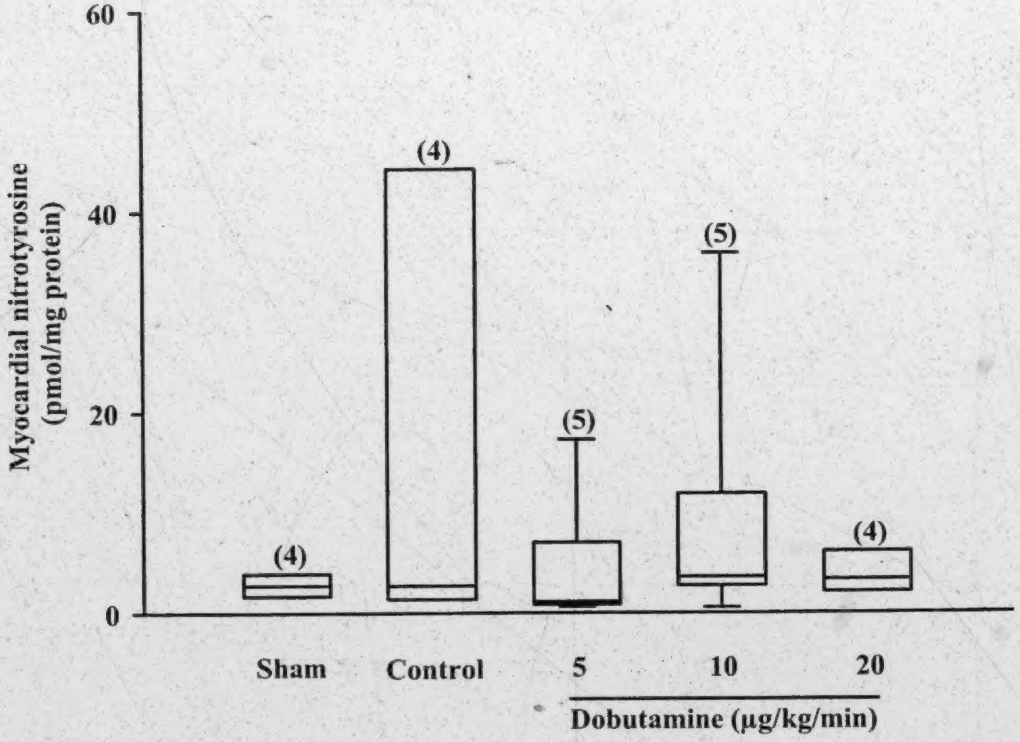


Figure 8-3: Left ventricular myocardial nitrotyrosine concentration in control (saline) and dobutamine-treated (5, 10 and 20 μg/kg/min) groups after 2h of hypoxia and 4h of reoxygenation. The sham-operated group had no hypoxia and reoxygenation. Detectable myocardial nitrotyrosine concentrations in each group are represented between parentheses. Median, 25th, 75th quartile range and 95% confidence interval are shown.



References

1. Martin-Ancel A, Garcia-Alix A, Gaya F, Cabanas F, Burgueros M, Quero J 1995 Multiple organ involvement in perinatal asphyxia. J Pediatr 127:786-793
2. Vento M, Asensi M, Sastre J, Garcia-Sala F, Pallardo FV, Vina J 2001 Resuscitation With Room Air Instead of 100% Oxygen Prevents Oxidative Stress in Moderately Asphyxiated Term Neonates. Pediatrics 107:642-647
3. Haase E, Bigam DL, Nakonechny QB, Rayner D, Korbutt G, Cheung PY 2005 Cardiac function, myocardial glutathione, and matrix metalloproteinase-2 levels in hypoxic newborn pigs reoxygenated by 21%, 50%, or 100% oxygen. Shock 23:383-389
4. Yasmin W, Strynadka KD, Schulz R 1997 Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. Cardiovasc Res 33:422-432
5. Szabo C, Salzman AL 1995 Endogenous peroxynitrite is involved in the inhibition of mitochondrial respiration in immuno-stimulated J774.2 macrophages. Biochem Biophys Res Commun 209:739-743
6. Cheung PY, Sawicki G, Wozniak M, Wang W, Radomski MW, Schulz R 2000 Matrix metalloproteinase-2 contributes to ischemia-reperfusion injury in the heart. Circulation 101:1833-1839
7. Hooper NM 1994 Families of zinc metalloproteases. FEBS Lett 354:1-6
8. Woessner JF, Jr. 1991 Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 5:2145-2154

9. Wang W, Schulze CJ, Suarez-Pinzon WL, Dyck JRB, Sawicki G, Schulz R 2002 Intracellular Action of Matrix Metalloproteinase-2 Accounts for Acute Myocardial Ischemia and Reperfusion Injury. Circulation 106:1543-1549

10. Sawicki G, Leon H, Sawicka J, Sariahmetoglu M, Schulze CJ, Scott PG, Szczesna-Cordary D, Schulz R 2005 Degradation of Myosin Light Chain in Isolated Rat Hearts Subjected to Ischemia-Reperfusion Injury: A New Intracellular Target for Matrix Metalloproteinase-2. Circulation 112:544-552

11. Romanic AM, Harrison SM, Bao W, Burns-Kurtis CL, Pickering S, Gu J, Grau E, Mao J, Sathe GM, Ohlstein EH, Yue TL 2002 Myocardial protection from ischemia/reperfusion injury by targeted deletion of matrix metalloproteinase-9. Cardiovasc Res 54:549-558

12. Borke WB, Munkeby BH, Halvorsen B, Bjornland K, Tunheim SH, Borge GI, Thaulow E, Saugstad OD 2004 Increased myocardial matrix metalloproteinases in hypoxic newborn pigs during resuscitation: effects of oxygen and carbon dioxide. Eur J Clin Invest 34:459-466

13. Lorch SA, Banks BA, Christie J, Merrill JD, Althaus J, Schmidt K, Ballard PL, Ischiropoulos H, Ballard RA 2003 Plasma 3-nitrotyrosine and outcome in neonates with severe bronchopulmonary dysplasia after inhaled nitric oxide. Free Radic Biol Med 34:1146-1152

14. Ischiropoulos H 1998 Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. Arch Biochem Biophys 356:1-11

15. Piazza AJ 1999 Postasphyxial management of the newborn. Clin Perinatol 26:749-65

16. Miura T, Muraoka S, Ogiso T 1998 Antioxidant activity of adrenergic agents derived from catechol. Biochem Pharmacol 55:2001-2006

17. White M, Ducharme A, Ibrahim R, Whittom L, Lavoie J, Guertin MC, Racine N, He Y, Yao G, Rouleau JL, Schiffrin EL, Touyz RM 2006 Increased systemic inflammation and oxidative stress in patients with worsening congestive heart failure: improvement after short-term inotropic support.Clin Sci (Lond) 110:483-9

18. Schulze CJ, Wang WJ, Suarez-Pinzon WL, Sawicka J, Sawicki G, Schulz R 2003 Imbalance between tissue inhibitor of metalloproteinase-4 and matrix metalloproteinases during acute myocardial ischemia-reperfusion injury. Circulation 107:2487-2492

19. Kleiner DE, Stetlerstevenson WG 1994 Quantitative zymography: detection of picogram quantities of gelatinases. Anal Bioche 218:325-329

20. Zweier JL, Kuppusamy P, Lutty GA 1988 Measurement of endothelial cell free radical generation: evidence for a central mechanism of free radical injury in postischemic tissues. Proc Natl Acad Sci U S A 85:4046-4050

21. Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB 1988 Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest 82:476-485

22. Tsao PS, Lefer AM 1990 Time course and mechanism of endothelial dysfunction in isolated ischemic- and hypoxic-perfused rat hearts. Am J Physiol 259:H1660-H1666

23. Hess ML, Manson NH 1984 Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol 16:969-985

24. Cohen MV 1989 Free radicals in ischemic and reperfusion myocardial injury: is this the time for clinical trials? Ann Intern Med 111:918-931

25. Huie RE, Padmaja S 1993 The reaction of no with superoxide. Free Radic Res Commun 18:195-199

26. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA 1990 Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 87:1620-1624

27. Beckman JS, Koppenol WH 1996 Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 271:C1424-C1437

28. Radi R, Beckman JS, Bush KM, Freeman BA 1991 Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 288:481-487

29. Radi R, Rodriguez M, Castro L, Telleri R 1994 Inhibition of mitochondrial electron transport by peroxynitrite. Arch Biochem Biophys 308:89-95

30. Packer MA, Murphy MP 1994 Peroxynitrite causes calcium efflux from mitochondria which is prevented by Cyclosporin A. FEBS Lett 345:237-240

31. Radi R, Beckman JS, Bush KM, Freeman BA 1991 Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. J Biol Chem 266:4244-4250

32. Hausladen A, Fridovich I 1994 Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. J Biol Chem 269:29405-29408

33. Villa LM, Salas E, Darley-Usmar VM, Radomski MW, Moncada S 1994 Peroxynitrite induces both vasodilatation and impaired vascular relaxation in the isolated perfused rat heart. Proc Natl Acad Sci U S A 91:12383-12387

34. Davidge ST, Ojimba J, McLaughlin MK 1998 Vascular function in the vitamin E-deprived rat: an interaction between nitric oxide and superoxide anions. Hypertension 31:830-835

35. Haynes RL, Folkerth RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, Volpe JJ, Kinney HC 2003 Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J Neuropathol Exp Neurol 62:441-450

CHAPTER 9

Conclusions and implications

There is little information on the cardiovascular effects of dobutamine, a β-adrenoceptor agonist, when it is used to treat myocardial dysfunction and hypotension in asphyxiated neonates. Using a neonatal swine model of hypoxia-reoxygenation, we examined the dose-response effect of dobutamine (5-20 μg/kg/min) on systemic and regional circulations and oxygen metabolism. Because of the potential interaction between dobutamine and the β-adrenoceptor on the platelets, we also tested the hypothesis that dobutamine used during reoxygenation after hypoxia in newborn piglets could cause platelet activation and aggregatory dysfunction.

Hypoxia and reoxygenation in the newborn piglets resulted in hypotension and myocardial dysfunction. Dobutamine improved the cardiac output with a significant increase at 20 μg/kg/min. This was associated with corresponding increases in stroke volume and systemic oxygen delivery. Pulmonary vascular resistance was significantly lowered in all dobutamine-treated groups. There were no significant differences in heart rate, systemic and pulmonary arterial pressures, systemic vascular resistance and systemic oxygen consumption. Apart from a transient tendency toward increased mesenteric blood flow and oxygen delivery in the group receiving 20 μg/kg/min dobutamine, there were no significant effects of dobutamine on carotid and renal circulations.

In regard to the platelets, as a result of hypoxia and reoxygenation, platelet counts decreased similarly in all groups. Platelet aggregation tended to deteriorate with a rightward shift of concentration-response curve in piglets receiving 10 and 20 μg/kg/min of dobutamine. The group of 20 μg/kg/min of dobutamine had higher plasma thromboxane concentration compared to baseline. There were no significant differences in the plasma concentrations of cAMP, cGMP or nitrotyrosine or MMP-2 and -9 activities between groups.

As mentioned previously, neonatal asphyxia can lead to multiorgan dysfunction including myocardial dysfunction and pulmonary hypertension. Using dobutamine in this clinical scenario would improve the cardiac output, increase the systemic oxygen delivery and attenuate the effect of asphyxia on the pulmonary vascular resistance. These benefits can be achieved without affecting other systemic and regional hemodynamic variables. We therefore suggest the use of 10-20 µg/kg/min dobutamine in the treatment of shock and hypotension in asphyxiated neonates.

However, clinicians need to be careful when using dobutamine under these circumstances. Based on the findings on the *ex vivo* platelets function, further studies are needed to examine if dobutamine may aggravate the platelet activation and aggregatory dysfunction. As asphyxiated neonates have an increased risk of hemostatic disturbance, the risk of dobutamine on the platelet pathology warrants further clinical studies.

After all, this is an animal study with recognized limitations. Although this is a controlled randomized study that included sham-operated animals as well, this study was not blinded. We used 100% oxygen for resuscitation according to the latest recommendations by the American Heart Association. Due to the current debate regarding the use of 100% oxygen for resuscitation, modifications in the concentration of inspired oxygen may be required in future studies. It will be interesting to study the effect of different oxygen concentrations on the hemodynamic responses of dobutamine infusion.

The study was also powered to examine the effect of dobutamine on cardiac index. We have seen modest changes in some of the other hemodynamic measurements such as pulmonary arterial pressure and mesenteric blood flow with dobutamine infusion. This could be due to small sample size and short duration of infusion. We also calculated some of the variables such as stroke volume index which can be measured more accurately using a Miller® pressure-volume catheter (Millar Instruments, Inc. Houston, TX). We started the dobutamine infusion at 2h of reoxygenation as per protocol, when all hypoxia-reoxygenation piglets developed significant hypotension and shock. It may be late in some animals as we observed variable responses towards the dobutamine infusion. Indeed, in the clinical practice, the inotropic therapy will be started when first clinical feature of shock and hypotension develop.

The common carotid artery supplies both the brain and extracranial structures. Therefore, the blood flow through the common carotid artery may not represent the actual blood flow to the brain. However, it could give an estimation of the cerebral circulation as there are reports showing good correlations between the common carotid arterial flow and cerebral blood flow. Placing a Transonic flow probe around the internal carotid artery might be more accurate in measuring the blood flow to the brain.

Although platelet aggregatory function *ex vivo* using whole blood has been validated, the *in vivo* environment might be different. *In vivo* studies such as bleeding time to confirm the finding are warranted. Finally, due to the technical difficulties, we assayed the plasma concentrations of mediators of the signaling/mechanistic pathways such as cAMP and cGMP instead of the intraplatelet (intracellular) concentration of these nucleotides. Measuring the intracellular concentrations of these mediators might highlight the mechanistic pathways of platelet activation and aggregatory dysfunction due to dobutamine infusion. There is a lack of literature, in the neonatal population, that examines the interaction of dobutamine and platelets. Human studies in asphyxiated neonate are required to examine the effect of dobutamine on platelet function.

Future studies with larger sample size to investigate the effects of super-high doses (>20 μg/kg/min) of dobutamine on the systemic and regional circulations would be very interesting. It is not known yet if dobutamine at super-high dose would have more α-adrenoceptor action and would increase the systemic arterial pressure accordingly. It would be also exciting to examine the effect of a vasopressor like dopamine in combination with dobutamine (inotrope). Dobutamine would increase the cardiac output while dopamine would probably increase the systemic arterial pressure. In fact, this is one of the most commonly used combination therapies in the management of hypotensive neonates.